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# THE NEW ZEALAND JOURNAL OF SCIENCE AND TECHNOLOGY

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## CONTROL MEASURES AGAINST THE CHEESE-MITES, *TYROLICHUS CASEI* OUDS. AND *TYROPHAGUS LONGIOR* GERV.

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The following account deals with the control of mites in cheese-stores. Literature dealing with control measures is reviewed. The use of waxes, dusts, and fumigants as a protection against the mites was tested. It was found that cheeses when waxed received some protection against mite attacks, but, contrary to general belief, it was found that mites can bore through the wax and thus destroy it as a covering. Dusts did not prove efficient barriers to mite attacks, as the high humidity factor, a prerequisite in cheese-storage, is a serious handicap to their use. Of the fumigants used—namely, ammonia, methyl bromide, and dichloroethyl ether—the latter was considered most suitable. It is effective against mites in very low concentrations, it is easily handled and applied, and finally, it is cheap to use. Dichloroethyl ether may be used in the vapour state for fumigation, but a more convenient method giving a more lasting effect is to apply it as liquid to the shelving in the curing-room (1 lb. per 1,000 cubic feet of room space or 1 lb. per 100 square feet of shelving). Alternatively, the cheeses may be stood on scale boards treated with dichloroethyl ether. Methyl bromide is effective as an acaricide, but it is expensive to use, and lacks the persistent properties of dichloroethyl ether. Ammonia is also an effective acaricide, but it is unsuitable largely owing to the fact that it is far too readily absorbed by the surrounding media.

### INTRODUCTION

WHILE the presence of cheese-mites in New Zealand has been known for many years, they were hardly regarded as a problem of first importance, because they were largely kept in check in bulk cheese-stores by low temperatures (45° F.). During the war period, however, when the shipping problem became acute, it seemed likely at one stage that large quantities of our primary produce, such as beef, mutton, butter, and cheese, would have to be stored in this country until such time as shipping could be

arranged to move it. It seemed almost certain that in such circumstances cheese, which was ordinarily held in cool stores as stated above, would have to be transferred to improvised stores without cooling facilities in order that more perishable products might be held in the stores previously taken up by the cheese. If such a course proved necessary, the rapid multiplication of mites in stores, in which a temperature range anywhere between 45° F. to 75° F. could exist, would present an important problem; consequently, a practical and efficient method of dealing with the situation became an urgent necessity.

Regarding the species of mites, *Tyrolichus casei* Ouds. and *Tyrophagus longior* Gerv., commonly found in cheese-stores in New Zealand, little need be said here since this aspect forms part of the subject-matter of a paper by Robertson (1A) (in manuscript).

### LITERATURE REVIEW

It would be beyond the scope of this paper to attempt to review all the literature relating to control measures; fortunately for our purpose, an excellent summary dealing with mites in stored products has been published by Solomon(2), and the writers feel they cannot do better than quote from the summary those parts which will give a wider understanding of the problem in hand, or, again, where necessary, refer in some detail to individual papers. In regard to the control of mites on cheese, Solomon(2) reviews the literature as follows:

Mites (*Tyrolichus casei* [Ouds.] and other species) are an essential ingredient contributing to the desired aroma of Altenburger cheese, and they are specially cultured for this purpose (Hase, 1929, reprinted in Zacher, 1933). But in general they are undesirable pests.

A useful short account of the life-history and economy of the mites infesting cheese was given by Eales (1917). Jary and Stapley (1937) gave a comparative account of two of the commonest cheese-mites, which had often been confused.

Eales (loc. cit.) and E. de Ong and Roadhouse (1922) stressed the importance of thorough cleanliness during the curing stage, and recommended the clearing-away of dirt, fragments of cheese, and infested cheeses (also Wallace, 1915). Wallace (loc. cit.) and Eales (loc. cit.) pointed out the need for guarding against the introduction of mites on flies and other insects or on boots and clothing.

E. de Ong and Roadhouse (1922) and Dustan (1937) stated that perfectly waxed cheese was free from attack. According to Wallace (1915), mites get in through loose binding or cracked rinds, and pressed cheeses are more resistant to attack because of the hard rind.

Maintenance of low temperatures as a means of avoiding mite damage in the curing stage was suggested by E. de Ong and Roadhouse (1922); Dustan (1937) investigated the killing of mites by freezing. Directions have been given for fumigation with sulphur, with carbon bisulphide and with hydrogen cyanide (E. de Ong and Roadhouse, 1922), with carbon bisulphide and with ammonia (Cranfield, &c., 1934), and with a methyl-bromide-carbon-dioxide mixture and an ethylene-oxide-carbon-dioxide mixture (Dustan, 1937). Instead of the usual dry brushing, Eales (1917) recommended disinfestation of cheeses by carbon bisulphide applied with a brush.

For disinfecting cheese-stores, the use of hot water or steam was discussed by Dustan (loc. cit.), and a 5 per cent. carbolic wash was recommended by Eales (loc. cit.). Wallace (loc. cit.) recommended the scrubbing of walls and woodwork with an emulsion of soft soap and kerosene.

HASE, A.: Zur pathologisch-parasitologischen und epidemiologisch-hygienischen Bedeutung der Milben. *Ztschr. f. Parasitenk.*, 1929, I, 4-5, pp. 765-821.

ZACHER, F.: Haltung und Zucht von Vorratsschädlingen. Handbuch der Biologischen Arbeits Methoden. Abt. IX, Methoden der Erforschung der Leistungen des tierischen Organisms. T. 7, H. 3, *Acarina*, 1933, pp. 574-92.

EALES, NELLIE B.: The life-history and economy of the cheese-mites. *Ann. Appl. Biol.*, 1917, 4 (1 and 2), 28-35.

- JARY, S. G., and STAPLEY, J. H.: Investigations on the insect and allied pests of cultivated mushrooms. IX. *Tyroglyphus dimidiatus* Herm. (*longior*) Gerv. A note on *Tyroglyphus longior* var. *castellani* (Hirst). The identity of *Tyroglyphus siro* L. (Gerv.). *J. S.E. Agric. Coll.*, 1937, 40, 119-33.
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- WALLACE, M.: Cheese-mites. *Agr. Gaz. N.S.Wales*, Sydney, 1915, 26 (8), 699-700.
- DUSTAN, G. G.: The effects of temperature and certain chemicals on cheese-mites. *Ent. Soc. Ontario, 68th Ann. Rept.*, 1937, 60-7.
- CRANFIELD, H. T.; ROEBUCK, A.; and STAFFORD, J. G. W.: The control of mites in cheese-stores. *J. Min. Agr.*, 1934, 41 (4), 347-52.

For the treatment of small quantities of cheese in a fumigation vault or tight container, Back and Cotton(3) recommend carbon bisulphide 10 lb., ethylene oxide 2 lb., or ethylene-oxide - carbon-dioxide mixture (one part by weight ethylene oxide to nine parts of carbon dioxide) 20 lb. per 1,000 cubic feet of space for a period of twenty-four hours. In regard to methyl bromide as a fumigant they stated: "It has remarkable powers of penetration and is undoubtedly the most efficient fumigant known for the treatment of warehouses filled with bagged commodities. As it does not kill quickly, it is useless in loosely constructed buildings where leakage is high." Muggeridge and Dolby(4) stated that dichloroethyl ether is extremely toxic to cheese-mites when applied to wood with which a mite-infested cheese came in contact. Robertson(1a) refers to the advantages of controlling mites at the source of infestation by adopting hygienic methods.

Cranfield *et. al.*(5) found *Tyroglyphus longior* the most prevalent cheese-mite in the Midlands, England. They gave the following four factors as essential in an acaricide:

- "(1) That it will kill a high percentage of the mites, including those in the crevices of the cheeses.
- "(2) That it shall be non-inflammable and in no way dangerous to use.
- "(3) That it shall be reasonably cheap.
- "(4) That it will not impart a flavour to the cheese or damage its market value in any way."

They pointed out that ammonia gas in suitable concentration is a reliable acaricide for the destruction of cheese-mites, and that in no case was any detrimental effect on the flavour of the cheese observed. In one trial, however, the odour of ammonium butyrate was observed to a slight extent: the coats of cheeses were wet and slimy, though ventilation reduced the wetness to some extent. They state that "ammonia gas at a concentration of 1:25 in a reasonably airtight storeroom has been found effectively to destroy cheese-mites in less than twelve hours' exposure, and kills of over 90 per cent. have been obtained in various experimental fumigations under commercial conditions."

Dustan(6) tested ammonia, pyrethrum fumes, sulphur dioxide, "Weevilcide," naphthalene, formaldehyde, a mixture of methyl bromide 6.8 per cent. and carbon dioxide 93.2 per cent., and a mixture of ethylene oxide one part and carbon dioxide nine parts. Of these, only the latter two gave satisfactory results. Ammonia at a concentration of 1:20 (sp. gr. 0.95,  $\text{NH}_3$  28.3 per cent.) killed all mites in exposed positions, but only about 30 per cent. kill on the under-surface next the floor. The cheese at first had a slight odour and a fairly marked taint in flavour, but both of these disappeared after twenty-four hours' exposure to the air.

He was of the opinion, however, that ammonia fumes were so irritating and penetrating that it would not be a practical fumigant to use. The two fumigants considered practicable were methyl-bromide - carbon-dioxide mixture, and ethylene-oxide - carbon-dioxide mixture. Regarding the former mixture, it was found effective used at rates varying from 8 lb. to 20 lb. per 1,000 cubic feet at temperatures of from 58° F. to 63° F. The effect of the gas on the cheese showed that unwaxed exposed portions of cheese picked up a decidedly unpleasant flavour which extended to about  $\frac{1}{4}$  in. below the surface. This foreign taste, however, completely disappeared after an exposure to the air of from thirty-six to forty-eight hours. The fumigated cheese was also fed to animals and no injurious effects were noted.

Ethylene-oxide - carbon-dioxide mixture, which is non-inflammable and non-combustible under ordinary conditions, was reported as having exceedingly good penetrating powers. It is not highly toxic to man, though proper precautions must always be used in handling it. It was used at the rate of 20 lb. per 1,000 cubic feet. It gave results almost identical with those obtained for methyl-bromide - carbon-dioxide mixture. It had the added advantage of not imparting even a temporary taint in flavour or odour. No toxicity tests were conducted with this material.

Searls *et al*(7) in a recent paper describe the use of methyl bromide as a fumigant for dairy factories for the control of such pests as cheese-skippers, cockroaches, cheese-mites, and rodents. This is a useful article dealing in a practical manner with the fumigation of buildings in different states of repair, and providing a table giving temperature, dosage, exposure, &c.

### POSSIBLE METHODS OF CONTROL

In the following account the authors have considered different possible methods of control under the headings—

- (A) Waxes.
- (B) Dusts.
- (C) Fumigants.

#### (A) WAXES

The waxing of cheese has been common practice, but was restricted in New Zealand before the war and was discontinued during the war period. Waxing was done mainly to reduce loss of moisture from the cheese, and any effect it may have had in protecting the cheese from attack by mites was incidental. If it could have been shown that a wax coating offered a valuable protection against mites, waxing might have become a necessity.

The waxes used were as follows:—

- (a) Paraffin wax.
- (b) Proprietary wax. Composition given as: paraffin wax, 57 per cent.; vaseline, 38 per cent.; rosin, 5 per cent.
- (c) Proprietary wax. Composition not given, but appeared to be ceresin with approximately 4 per cent. rosin.
- (d) Butterfat.

Loaf cheeses weighing approximately 10 lb. each were manufactured in the Dairy Research Institute factory, treated with wax when fourteen days old, and then shipped to Nelson for the experiment. Twenty-five cheeses were used, thus allowing each treatment to be replicated five times. Each set of five cheeses was from the same vat. The experiment was carried out in a small room in which the temperature varied according to outside

conditions, though during the winter months a small radiator was used to prevent the temperature dropping too low. The mean maximum and minimum temperatures and humidities and their standard deviations were recorded and are given in Table 1. These results were computed from weekly mean maximum and minimum temperatures and humidities. The humidity was maintained at a reasonably high level by pouring water over the floor of the room when necessary. The experiment was commenced on 27th November, 1942, and continued till 24th August, 1943, a period of 270 days. The cheeses were placed on a flat table surface in the experimental room and colonies of mites were scattered over the surface of the table, with the result that all the cheeses became mite-infested. The cheeses were weighed at regular intervals to determine the effect of the different waxes in controlling loss of moisture from the cheeses.

TABLE 1. PERCENTAGE LOSS IN WEIGHT OF WAXED AND UNWAXED CHEESES

Mean maximum temperature	..	..	64.3	6.5 F.
Mean minimum temperature			57.0	4.8 F.
Mean maximum relative humidity	..		83.9	6.5 per cent.
Mean minimum relative humidity	..		72.2	7.1 per cent.
Age at first weighing: 21-25 days.				

	14 Days	33 Days	62 Days	154 Days	188 Days	270 Days
(a) Paraffin wax	0.1	0.8	1.9	4.6	5.0	6.4
(b) Proprietary wax	0	0.7	1.3	3.2	4.2	4.6
(c) Proprietary wax	0.1	0.6	1.1	2.9	3.7	3.9
(d) Butterfat	1.0	2.6	4.2	7.3	8.0	9.4
(e) Unwaxed	1.7	2.8	4.8	8.1	8.7	10.4

The two proprietary waxes gave a rather better retention of moisture than the paraffin wax, possibly due to the fact that these waxes were more plastic than the paraffin wax and so less liable to develop cracks.

The results of an examination at the end of the 270-day period showed the following results of mite attack:—

- (a) Heavily infested. Wax covering perforated and eaten away in large sections.
- (b) Heavily infested. Wax covering perforated and honeycombed by mites.
- (c) Heavily infested. Wax covering being bored through and eaten out in large holes.
- (d) Heavily infested. Fat covering perforated and honeycombed by mites.
- (e) Heavily infested.

#### Discussion

All the waxed cheeses were subjected to a severe test against mite attacks and none was resistant to these attacks—the mites attacked the wax covering and gradually bored through it; the portions of wax attacked had the appearance of numerous individual cells penetrating it at right angles to the surface; the cells themselves contained individual mites. That the wax offered considerable resistance to the mite penetration seemed clear, as the control cheeses in every case showed far more damage than the waxed cheeses. Practically all of the mites found in the cheeses throughout the greater part of the experiment were *Tyrolichus casei*.



## (B) DUSTS

We have not come across any records in the literature concerning the use of dusts against mite-infested cheeses. Solomon(2) refers to the use of hydrated ferrous phosphate dust for the control of mites in grain, and the use of toxic dusts such as copper carbonate, calcium cyanide, ground sulphur, and magnesium carbonate, none of which is considered suitable for use where food materials are involved. The writers took the view that certain mineral dusts might prove useful as barriers to the spread of mites, in view of the fact that they would be cheap to obtain and easy to use if they proved successful.

The experiments with dusts were carried out in the same room and under the same conditions of temperature and humidity as were the experiments with waxes, though the dusting experiments took a much shorter time to complete. The method of preparing and setting out the experiments was as follows: 10 lb. loaf cheeses were dipped in wax (proprietary wax C) and then cut parallel with the ends so as to divide each cheese into two equal portions. After cutting, the cheeses were again dipped so as to seal the edges of the cut surfaces and leave only the freshly cut portion exposed to mite attacks. In experiment 1 the cut surface was heavily dusted and placed face down on a clean table surface and the waxed surfaces were then coated with dusts. In experiments 2 and 3, in order to spread the dusts as evenly as possible, a hollow 9 in. cylinder was first placed on the table and a coating of dust applied, the cheese (6½ in. to 7 in. diameter) was then placed inside the cylinder and again dust applied, with the result that a circular dust barrier was spread on the table surface about 1 in. wide around the cheese. The dusts used were dolomite, magnesite, serpentine, sulphur, and calcium carbonate, all of which had been passed through a 150-mesh sieve. After the experiments were set, a heavy infestation of mites was placed on the table with ample food to maintain a strong colony for the duration of the experiment. The arrangement of the treated and untreated cheeses was as follows:

## EXPERIMENT 1.—DURATION: 30 DAYS

Dolomite dusted.	Untreated.	Magnesite dusted.	Untreated.	Dolomite dusted.	Untreated.	Magnesite dusted.
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## EXPERIMENT 2. - DURATION: 60 DAYS

Dolomite dusted.	Serpentine dusted.	Magnesite dusted.	Sulphur dusted.	Dolomite + Serpentine.	Untreated.	Magnesite dusted.
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## EXPERIMENT 3. - DURATION: 60 DAYS

Dolomite dusted.	Magnesite dusted.	Sulphur dusted.	Untreated.	Serpentine dusted.	Calcium carbonate dusted.
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*Discussion*

The dusts are apparently non-toxic and their action is purely mechanical. It was observed that numbers of mites which attempted to cross the barrier were finally brought to rest by the weight of dust particles adhering to them and that they died before escaping from the dust. Other mites, however, succeeded in crossing the dust barrier, so that, while the treatment delayed the mite invasion, the treated cheeses all eventually became infested, though not so rapidly as the untreated cheeses. It seems clear that when mites are present in large numbers dust barriers of the kind used would be of relatively little value for protecting a product such as cheese. The conditions under which dusts would probably be most effective—i.e., low humidity—are the conditions least suitable for storing cheese.

## (C) FUMIGANTS

Solomon(2) has listed a wide range of chemicals which have been used as fumigants against mites infesting various products. The list includes the following: hydrogen sulphide, ammonia, hydrogen cyanide, nitrous oxide, sulphur, sulphurous slag, chlorine, carbon dioxide, carbon bisulphide, paradichlorobenzene, trichloroethylene, carbon tetrachloride, naphthalene, benzene, chloropicrin, pyridine, aniline, monomethylaniline, formaldehyde, ethylene oxide, methyl bromide + carbon dioxide, methyl salicylate, ethyl acetate + carbon tetrachloride, ethylene dichloride (dichloroethane), "Areginal" (methyl formate).

*Ammonia*

It was obvious that many of the chemicals listed would not, for different reasons, be suitable for the fumigation of cheese. The writers decided first to use ammonia, since it was readily obtainable and was relatively cheap, and also in view of the work already referred to, which indicated that its possibilities were promising.

*Experimental.*—In preliminary laboratory experiments mites were placed in a petri dish in a large earthenware jar (6 l.) into which a known quantity of ammonia gas was introduced. The jar was then sealed and placed in a thermostatically controlled cabinet. The results were somewhat variable, due to lack of any means of circulating and mixing the air and ammonia and to lack of control of humidity. For this reason the results have not been reproduced in detail. It was possible to show, however, that ammonia gas was highly toxic to mites, an exposure for one hour to a concentration of 30 mg. per litre, giving 100 per cent. mortality.

*Effect of Ammonia on Cheese.* Small-scale trials were made to determine the effect of ammonia vapour on the flavour of cheese. A series of loaf cheeses were enclosed in bell-jars each containing a dish of dilute ammonia solution of such concentration as to give the desired partial pressure of ammonia in the air round the cheese. It was found that the outside of the cheese acquired an ammoniacal odour and an unpleasant soapy taste. The inside of the cheese was unaffected. The depth of penetration was readily observed with coloured cheese in which the outer layer became discoloured. The depth of the discoloured layer varied according to the concentration of ammonia and time of exposure. With the minimum concentration and time of exposure necessary to kill cheese mites—e.g., 10 mg. l. for twenty-four hours or 25 mg. l. for three hours—the depth of the affected layer was less than  $\frac{1}{8}$  in. and so would not cause appreciable damage to the cheese.

*Large-scale Trials.* The first trial was made in a room measuring 22 ft.  $\times$  11 ft.  $\times$  10 ft. (cubic capacity, 2,420 cubic feet). The walls and floor were of concrete and the ceiling of wood, and the room was fitted with shelving on which there were a number of cheeses. Windows and doors were closed, but no special precautions were taken to make the room gastight. Ammonia from a cylinder was admitted through a hose passed through a drainage opening in an outer wall. The cylinder was placed on scales so that the weight of gas admitted could be determined. Six pounds of ammonia was introduced, giving a dosage of  $2\frac{1}{2}$  lb. per 1,000 cubic feet (= 40 mg./l.). The atmosphere was sampled through a tube at the opposite end of the room about 3 ft. from the floor. The samples were taken in evacuated separating funnels of about 250 ml. capacity. Before this was done the tube was flushed out by aspirating a quantity of air through it.

Ammonia in the sample was determined by introducing a quantity of standard acid which was shaken round and then rinsed out into a flask and titrated. Results obtained are given in Table II.

TABLE II.—TABLE SHOWING RESULTS OF IMMEDIATE SAMPLING AND AT INTERVALS AS STATED FOLLOWING RELEASE OF AMMONIA SUFFICIENT TO GIVE A CONCENTRATION OF 40 MG. PER LITRE

Time after Ammonia Admission completed.	Concentration (mg./l.)
Hr. min.	
0 0	10
1 30	6
5 0	Nil

Examination of cheeses showed that only a very incomplete kill of mites had taken place.

A second trial was made in a different room which was chosen for the purpose. This was an isolated building with brick walls and concrete floor and ceiling. It had one door but no windows. There were a number of ventilators, which were covered with cardboard before commencing the fumigation. The dimensions of the room were 27 ft. 6 in.  $\times$  15 ft.  $\times$  8 ft. 6 in., giving a cubic capacity of 3,506 cubic feet. At one end of the room a stack of 150 cheese-crates was erected to simulate a stack of crated cheese. Cardboard cylinders inside the crates were used to restrict diffusion to the amount which would occur in a stack of crated cheese. The crates were stacked three tiers high and the top of the stack was about 18 in. below the ceiling. Glass tubing was arranged to permit sampling from four points of the stack: at the front and the back of the stack 6 in. above the floor and at corresponding points at the top of the stack. The tubes led out under the door, which was sealed with adhesive tape before commencing the fumigation. The ammonia was admitted through a rubber hose passing through a hole in the door. A dosage of 2½ lb. per 1,000 cubic feet (40 mg./l.) was again used. Admission of ammonia took ten minutes. Samples were taken at intervals from the four points by the method already described. Results are given in Table III.

TABLE III

Time after Ammonia Admission completed.	Concentration of $\text{NH}_3$ in Air (mg./l.).			
	Front Top.	Front Bottom.	Rear Top.	Rear Bottom.
Hr. min.				
0 5	3.5	0.5	8.5	0.4
0 30	3.0	1.0	2.0	0.5
1 50	2.5	0.5	0.5	Nil
19 0	Nil	Nil	0.3	Nil

The results show a rapid loss of ammonia due either to diffusion or absorption. Some laboratory experiments were made to determine extent of absorption of ammonia by brick or concrete. An apparatus was set up as shown in the diagram (Fig. 1).

A 20 l. aspirator jar (A) was connected by glass tubing leading from its neck to the tubulure of an inverted bell-jar (B). The neck of the bell-jar was connected with a rubber bellows (C), which in turn was connected to the tubulure of (A). The bellows was operated by a crank mechanism (D) driven by a motor through reduction gearing. The rate of air circulation was about 3 l. per minute. Samples of absorbing material were placed in the bell-jar, which was closed by a glass plate. To introduce a known

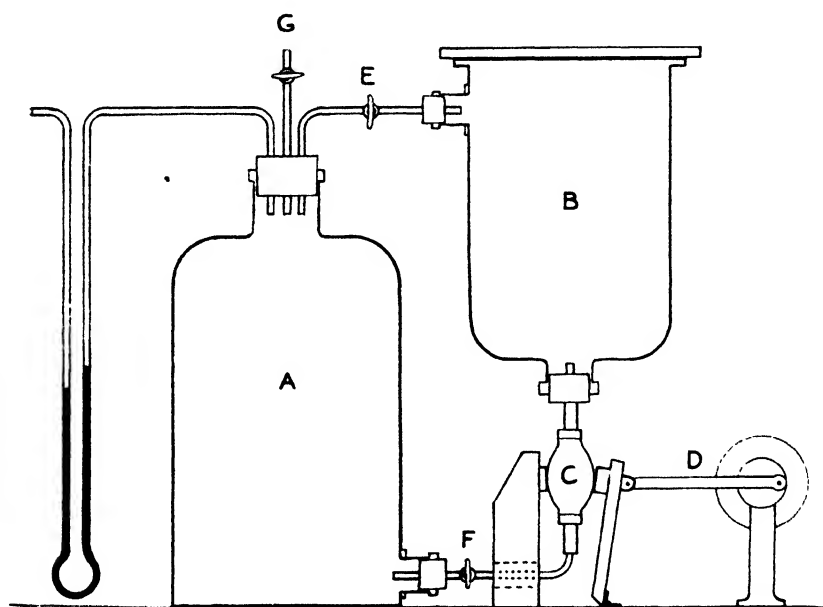


FIG. 1

quantity of ammonia, the vessel (A) was isolated from the rest of the apparatus by closing the taps (E) and (F). Pressure in (A) was reduced to the desired extent by evacuation, and ammonia was then admitted from a cylinder through tube (G) till atmospheric pressure was again reached. The concentration of ammonia in the system was calculated from the manometer readings and the volume of the apparatus (31 l.). Samples of gas were withdrawn as required through the tube (G). In one experiment a brick (9 in.  $\times$  4½ in.  $\times$  3 in.) was placed in the vessel (B), while in another a concrete roofing-tile (16 in.  $\times$  9 in.) was used. In a third experiment a loaf cheese was placed in the apparatus. The brick and tile were each washed, and then dried for twenty-four hours at room temperature before being

placed in the apparatus. Circulation of air in the apparatus was not commenced till after the object had been introduced and the bell-jar closed. Results are given in Table IV.

TABLE IV

Absorbing material	Control.	Brick	Concrete Tile.	Cheese.
Ratio surface of material Volume of apparatus (cm. - 3)	Nil	0.032	0.066	0.037
Concentration $\text{NH}_3$ added (mg. l.)	40	40	40	145
Concentration in mg. l. after lapse of -	Concentration found in mg. l.			
Hr. min.				
0 15	.	.	.	63
0 30	.	.	.	39
0 45	36.5	12.5	15.8	.
1 0	.	.	.	28
1 15	.	9	12.5	.
1 45	36	..	.	.
2 0	.	.	.	22
3 0	35.7	.	..	20
3 15	.	7	..	.
3 50	.	.	7.5	.
6 0	.	6	.	.
6 50	.	.	5	.
20 0	.	.	.	12
21 0	36	..	.	.
23 0	.	.	3	.
24 0	.	.	.	.
70 0	.	5.5	.	.

*Discussion.*—The control experiment with no absorbing material in the system showed that losses due to absorption were small and that leakage losses were negligible. Both brick and concrete absorbed most of the ammonia in the apparatus within a few hours. The cheese also absorbed ammonia rapidly. A further experiment with a brick which had been painted all over with oil-bound water-paint gave similar results to that with untreated brick.

The ratio of area of walls, floor, and ceiling to room volume for the store used in the second large-scale trial was  $0.015 \text{ cm.}^{-1}$ , as compared with  $0.032 \text{ cm.}^{-1}$  for the laboratory experiment using brick. Absorption of ammonia by the materials of which the room was built would therefore account for part of the losses found. The remaining losses must be attributed to leakage or diffusion. The rate of absorption of ammonia by cheese was very rapid, so that in a store containing a quantity of cheese the losses would be considerably greater than in an empty room.

From these results it was concluded that the production and maintenance of an effective concentration of ammonia in a cheese-store was impracticable, and work with ammonia was accordingly discontinued.

*Methyl Bromide*

Methyl bromide as a fumigant against mites in cheese-stores has already been referred to. It is a stable liquid with a b.p. of 4.5° c., sp. gr. 1.732. It vaporizes rapidly on exposure to air, and because it is at least three times heavier than air it tends to fill a room in the same manner as water would fill it. Commercially it has wide use as a fire-extinguisher, for which purpose it is put in small metal cylinders. It is used also in the technique of refrigeration. Methyl bromide is toxic to man, but what exactly constitutes a toxic dose is not known, though it has been stated that exposure for one hour to concentrations of 0.4 per cent. to 0.8 per cent. would be likely to prove fatal. Again, there is a possibility that it might act as a cumulative poison, though the literature on this point is conflicting. Headaches, vomiting, uncertain gait, tiredness, double vision, and mental disorders are some of the symptoms of methyl bromide poisoning. It is inappropriate to deal with the medical aspect of this subject here, but it seems desirable, on the other hand, to draw attention to some of the hazards to health which might result from any carelessness in its use.

As a precaution, a good gas-mask should always be worn when fumigating with methyl bromide. A detailed account of the precautions necessary when fumigating dairy products and plant has been given by Searls *et. al.*(7).

*Experimental.*—The laboratory experiments with ammonia gas showed that it was not possible to secure reproducible results unless the apparatus had proper means of circulating and mixing air and fumigant and of controlling the humidity. After a search for a suitable design, it was decided to use an apparatus of the type described by Page and Lubatti(8).

The principal components of the apparatus were a large stainless-steel metal cylinder reservoir (A) (Fig. 2) of approximately 134 l. capacity, with a removable lid (B) held in place by wing nuts (L), a vacuum gauge (C), a motor-driven ball-bearing fan (D) for circulating and mixing the cylinder contents, and four small fumigation cells (M) each cell approximately 450 ml. capacity, in which the material to be fumigated could be placed. The apparatus was operated as follows: the cylinder was evacuated through nozzle (F), controlled from valve (H), and the required fumigant and water vapour were then taken into the cylinder through the same connections. The gas was then circulated by the fan (D), which has a lead to the bottom of the cylinder. By closing stopcock (J) and opening valve (G) (the latter was seated in the lid) the cylinder contents were drawn through pipe (K) and forced to the bottom of the cylinder and thus continually circulated and mixed. Mites in petri dishes were placed in fumigation cells (M), which were sealed to exterior influences by means of plate-glass disks (O) lightly smeared with vaseline at the edges and pressed down on to the flanged edge, and held in place by means of metal spring clips (R): stopcocks (J) and (P) were opened, as also were the stopcocks (N), depending on whether one or more of the cells was to be used, while valve (G) was closed, with the result that the fumigant was drawn through the cells, through stopcock (J), through fan (D), and so on to the bottom of the cylinder. In order to keep the contents of the cylinder well mixed and to avoid undue use of the fan

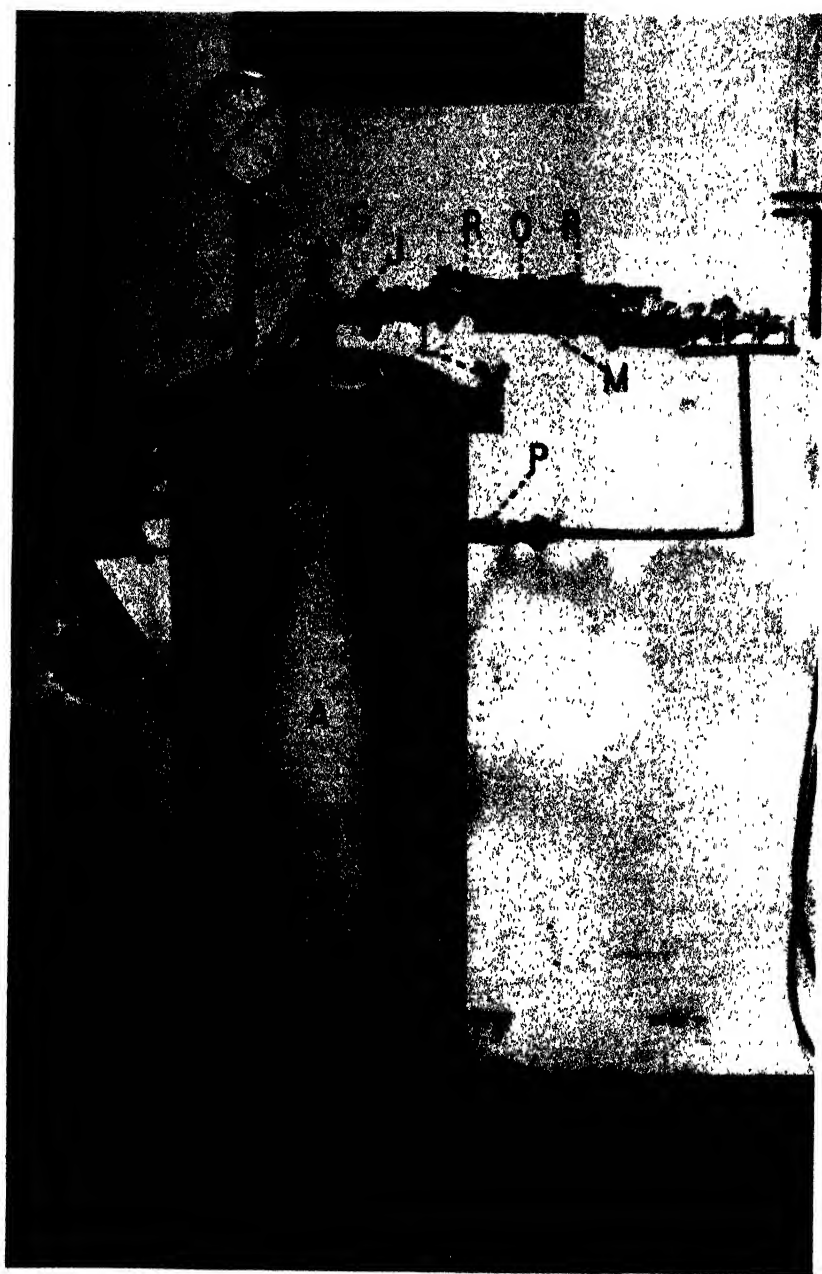


FIG. 2A

Figs. 2A and 2B.—Front and top views respectively of apparatus used for fumigating experiments. A, stainless-steel cylinder; B, lid; C, vacuum gauge; D, ball-bearing fan; E, motor for driving fan; F, nozzle through which the cylinder is evacuated and filled; G, wheel controlling valve seated in lid; H, valve control for nozzle F; J, stopcock; K, bent pipe connection; L, wing nuts for holding lid; M, metal cells for holding material being fumigated; N, stopcocks controlling passage of gas to cells M; O, plate-glass disk; P, stopcock; R, metal spring clip.

and motor, a time switch was employed which permitted the motor to switch in at fixed intervals. The factors controlled during operation of the apparatus were time, temperature, concentration of fumigant, and humidity, the latter being checked by means of the silver-mirror method at the end of each experiment.

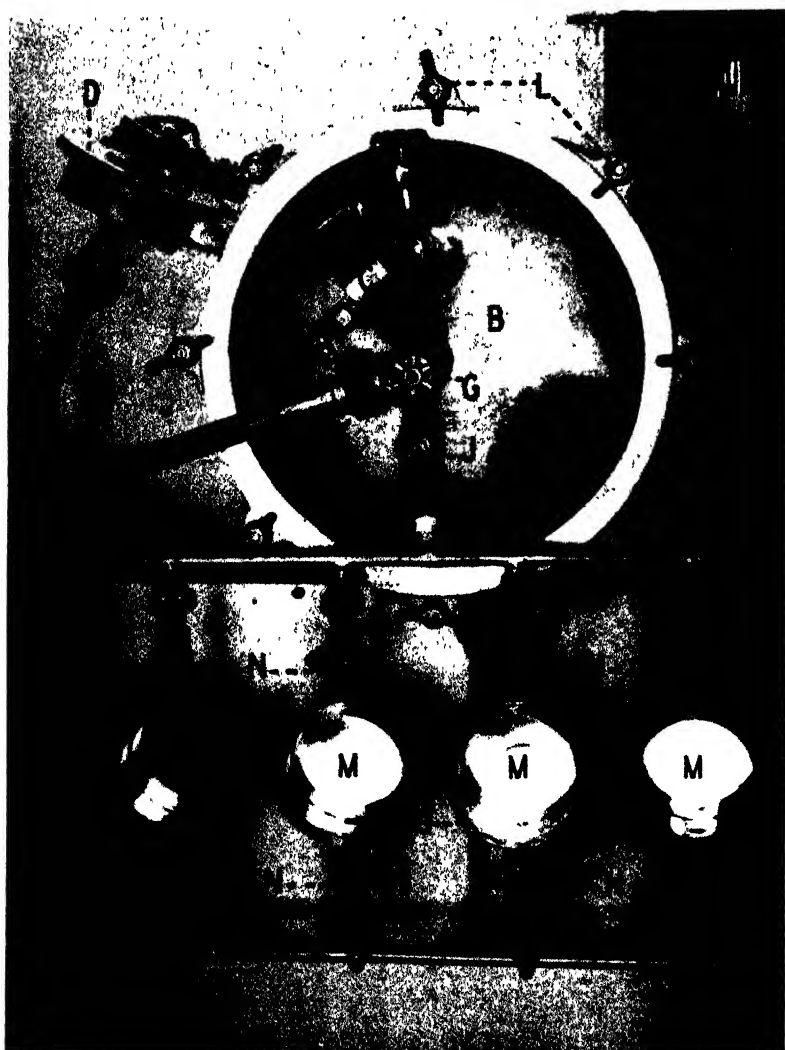


FIG. 2b

It was not feasible to make mortality counts immediately following exposure of the mites to the fumigant because of a time lag before the full effects of the fumigant were felt. The following procedure was therefore adopted. The petri dishes containing the mites were removed from fumigation cells (M); random samples of approximately twenty mites were



counted from the petri dishes into small cells (A) (Fig. 3), of the kind devised by Robertson(1). The cells for these experiments were made by boring a tapered hole through a wooden block  $\frac{1}{8}$  in. thick. The base was covered with porous paper, while the top was sealed by a rubber washer (B) and a glass square (C) held firmly in place by two spring clips (D). Mites were placed in the cell through the hole in the centre of the glass square, which was then closed by means of a small wooden plug (E). The cells were then placed in a desiccator jar, in which the relative humidity was kept at 80 per cent. by means of a saturated sodium chloride solution and held thus for a period of twenty-four hours at a temperature at which the fumigation portion of the experiment was carried out. At the end of the period the contents of the cells were counted and recorded as shown in the tables.

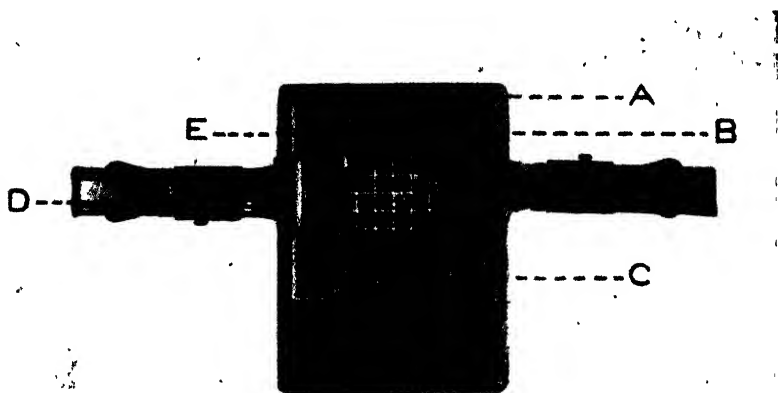


FIG. 3.—Cells used for holding mites following fumigation treatment. A, wooden block,  $1\frac{1}{8}$  in.  $\times$   $2\frac{1}{8}$  in.  $\times$   $\frac{1}{8}$  in.; B, rubber washer; C, glass square; D, spring clip; E wooden plug.

Using the apparatus already described, the laboratory procedure for testing the effect of methyl bromide on the mites was as follows: the methyl bromide vapour was measured volumetrically over a saturated solution of sodium chloride and then drawn into the cylinder, together with the required amount of water vapour, and air pressure within the cylinder was adjusted to the external pressure. By means of the fan the cylinder contents were thoroughly mixed and a sample of the mixture was then drawn off to check by analysis the methyl bromide concentration, using the method described later. Samples of mites for treatment were scraped from a cheese surface and placed in position for fumigation, but before fumigation commenced control samples were counted out into cells (A)

(Fig. 3). At the end of a twenty-four-hour period, following fumigation, counts were made and percentage mortality computed as described by Bliss(9). Table V gives results of two series of experiments.

TABLE V

Fumigant: Methyl bromide. Temperature: 68 F.  $\pm$  1. Relative humidity: 80 per cent.  $\pm$  3 per cent. Time of exposure of mites to fumigant: 4 hours.

Series.	Experiment No.	Milligrams per Litre	Number of Mites used.	Number of Mites survived.	Percentage Mortality.		Probit Kill
					Untreated.	Treated.	
1	1	11	160	38	0	76.2	5.71
	2	10.6	160	41	0	74.3	5.65
	3	10.3	160	26	2.5	83.3	5.96
	4	9.9	160	40	0	75	5.67
	5	9.5	160	72	0	55	5.12
	6	9.1	160	120	0	25	4.32
	7	8.7	160	143	0	10.6	3.75
	8	8.6	160	135	5	11.1	3.77
	9	8.4	160	132	7.5	10.8	3.76
2	1	12.3	140	29	0	79.2	5.81
	2	11.9	120	43	10	60.1	5.25
	3	11.5	120	74	0	38.3	4.70
	4	10.5	160	114	2.5	26.9	4.38
	5	10.2	160	134	2.5	14.1	3.92

Comparing series 1 with series 2 in Table V, a greater concentration of methyl bromide is required to kill in the latter series as compared with the former. Whatever the true explanation of this difference may be, one point does seem clear—namely, that environment plays some part in mite resistance to a fumigant. This point will be discussed more fully when dealing with dichloroethyl ether. Dealing with anomalies, as in series 1, experiments 3, 8, and 9, environment may again have played a part, but the relative ages of the mites comprising the sample might also be equally responsible for the differences. The writers are convinced that for more exact results in investigations of this kind the mites should be reared under controlled conditions so that age and environment preceding treatment are known.

The dosage mortality curves (Bliss(9)), calculated from the data in Table V, are shown in graph form in Fig. 4. In Table VI the values for  $\chi^2$  show that the observed points do not vary significantly from the expected points on the regression lines, and, again, the value for  $t$  indicates that the differences of slope of the lines is not significant.

TABLE VI

Series.	Difference in $b$ the Slope of the Lines						
	$n$	$\chi^2$	P.	$b$	Variance of $b$	Pooled Variance.	$t$ .
1	7	0.0038	0.99	20.752	1.015	1.543	0.316
2	3	0.003	0.99	20.368	2.775	..	..

There is relatively little difference in the toxic effect of methyl bromide on the two common cheese-mites, *Tyrophagus longior* Gerv. and *Tyroliehus casei* Ouds., as the results in Table VII will show. Both species of mites in each test were treated by the same concentration of the fumigant at the same time.

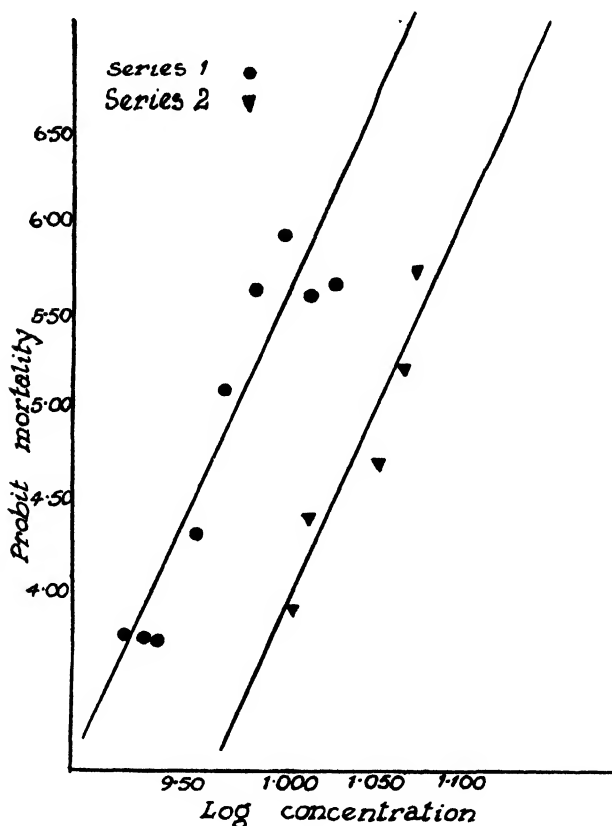


FIG. 4.—Graph showing dosage mortality curves.

TABLE VII  
Fumigant: Methyl bromide

Experiment No.	Milli-grams per Litre.	Number of Mites used.	Number of Mites survived.	Percentage Mortality.		Temperature.	Relative Humidity.	Time (Hours).	Mite Species.
				Untreated.	Treated.				
1	11.5	240	93	14.5	54.7	68	80	4	<i>casei</i>
2	11.8	159	5	0	96.9	68	32	4	<i>longior</i>
		159	20	2.5	87.0	68	32	4	<i>casei</i>
3	11.7	159	19	0	88.1	68	36	3½	<i>longior</i>
		159	25	5.2	82.6	68	36	4	<i>casei</i>
4	11.7	160	14	7.5	90.5	68	78	4	<i>longior</i>
		157	8	5	94.7	68	78	4	<i>casei</i>
5	10.7	159	36	0	77.4	68	83	4	<i>longior</i>
		159	101	5.1	33.1	68	83	4	<i>casei</i>

In experiment 3 the mortality figure for *longior* is lower than it should be, because exposure to the fumigant was half an hour less than it should have been. With the exception of experiment 4, the results indicate that *casei* is slightly more resistant to the fumigant than *longior*.

**Determination of Methyl Bromide.**—The method of Stenger, Shrader, and Beshgetoor(10) was used. The sample of gas was taken in an evacuated vessel of 250 ml. to 500 ml. capacity. Two millilitres of ethanolamine were introduced through the stopcock of the vessel by means of a capillary tube. After fifteen minutes, when hydrolysis of the methyl bromide by the ethanolamine was complete, the sampling vessel was washed out with water, the solution acidified with nitric acid, and the bromide determined by the Volhard method. Determination on a weighed quantity of methyl bromide gave 100 per cent. recovery. In a further experiment, using the apparatus described previously (Fig. 1), a quantity of methyl bromide vapour sufficient to give a concentration of 100 mg./l. was introduced into the apparatus. A sample taken after forty-five minutes showed 85 mg./l., and one taken five days later, 84 mg./l.

**Absorption of Methyl Bromide by Cheese.**—Absorption of methyl bromide by cheese was investigated in the same manner as absorption of ammonia. Results are given in Table VIII.

TABLE VIII

(CH <sub>3</sub> Br introduced (calc.)	..	..	..	29 mg. l.
Concentration (CH <sub>3</sub> Br found after forty-five minutes	..	..	..	15.0 mg./l.
Concentration CH <sub>3</sub> Br found after three hours	..	..	..	15.0 mg./l.
Concentration (CH <sub>3</sub> Br found after nineteen hours	..	..	..	15.6 mg. l.

The results showed that, as compared with ammonia, the absorption of methyl bromide by cheese was small.

**Large-scale Trials.**—A trial fumigation was carried out in the same room as was used for the second trial with ammonia. The same stack of cheese-crates was present and arrangements for withdrawing samples were the same. A few mite-infested cheeses were placed in the stack of crates. Methyl bromide from a cylinder was admitted through a hose and allowed to run on to the floor and evaporate from there. Results are given in Table IX.

TABLE IX

Methyl bromide introduced : 4½ lb. = 1.2 lb. per 1,000 cubic feet = 19.5 mg. l.

Time after Admission of CH <sub>3</sub> Br completed.	Concentrations found (mg l.).			
	Stack Front.		Stack Rear.	
	Top.	Bottom.	Top.	Bottom.
Hr. min.				
0 20	1	70		
1 0	3	72	3	11
5 0	5	6	5	6
18 0	0.5	0.7	0.6	1.3

The results in Table IX showed that there had been serious stratification of the vapour and that when, after five hours, the vapour had diffused uniformly through the stack its concentration had fallen too low to be effective. A considerable proportion of the mites present on the cheeses were found to be still alive after the fumigation.

A second fumigation was carried out later. Six cheeses heavily infested with mites were placed in crates at various parts of the stack. The methyl bromide was led from the hose into a shallow pan, while a fan was arranged to draw air over the pan and blow it vertically upwards. Results obtained were as follows:—

TABLE X

Methyl bromide introduced : 15 lb. = 4.3 lb. per 1,000 cubic feet = 68 mg.  $\text{l.}$

Time after Admission of $\text{CH}_3\text{Br}$ completed.	Concentrations found (mg $\text{l.}$ ).			
	Stack Front.		Stack Rear.	
	Top.	Bottom.	Top.	Bottom.
Hr. min.				
0 20	13	17	15	12
0 50	22	12	17	18
2 0	22	25	..	12
6 30	4	24	3	4
9 45	4	27	2	5
23 0	Nil	Nil	.	Nil

The figures above indicate that uniform distribution of vapour throughout the stack had been secured. The concentration reached was very much below that expected from the quantity of methyl bromide introduced, but was sufficiently high for the first two or three hours to give an effective kill of mites. This was confirmed when the cheeses were examined. No living mites were found and it was evident that a sufficient concentration had been reached even under the cheese and in cracks in the cheese.

In order to determine whether a complete kill of eggs had also been obtained, one cheese was crated and wrapped in cellophane, the edges of which were secured with adhesive cellulose tape before removing the crate from the room. This covering should have removed any chance of reinfestation. The cheese was examined eight weeks later and a colony of live mites was found on a small area under the cheese. Evidently a number of eggs in this area had escaped destruction.

*Discussion.*—It was concluded from these experiments that fumigation of a store with methyl bromide at the rate of 4 lb. per 1,000 cubic feet would give a useful control of mites, but that, while valuable as an emergency measure for treating a store filled with crated cheese, the method would be too costly for use in factory curing-rooms. The cost, at New Zealand retail prices, of the methyl bromide used in the second treatment of the experimental store was £24. While methyl bromide could be secured more cheaply by bulk importation, the method would still be expensive. In addition, the construction of many factory curing-rooms is such that they could not be rendered gastight and excessive losses of fumigant would occur.

#### *Dichloroethyl Ether*

Dichloroethyl ether has been used as a soil fumigant against wireworms by Campbell and Stone(11), by Campbell(12), and by Pepper(13), against plum curculio by Snapp(14), and against the woolly aphid by Underhill and Cox(15). It has also been used as a glasshouse fumigant by Willcoxon

and Hartzell(16). Muggeridge and Dolby(14) found dichloroethyl ether to be extremely toxic to cheese-mites, and showed that if the wood that cheese stands on was treated with it, it remained toxic over a considerable period of time. More recent research both in the laboratory and in cheese-stores fully confirms its value as an important acaricide.

The vapour of dichloroethyl ether is toxic to human beings, and as it is important that users should have a knowledge of the hazards involved, the following data on its toxicity have been summarized from a paper by Schrenk, Patty, and Yant(17) in Table XI.

TABLE XI.—TOXICITY OF DICHLOROETHYL ETHER

Volume Concentration (Percentage).	Milligrams per Litre.	Pounds per 1,000 Cubic Feet.	Effect on Guinea-pigs	Warning Properties.
0.035	0.2	0.01	No serious response after 12 hours' exposure	Easily noticeable odour. Only slightly offensive and practically free from irritation of eyes and nose.
0.01-0.02	0.5-1.0	0.03-0.06	No serious response after 60 minutes' exposure. Disturbance of respiration after five or six hours' exposure. Death after twelve hours' exposure	Slightly nauseous odour and slight irritation of eyes and nose.
0.05-0.10	3-6	0.2-0.4	Ten minutes' exposure caused slight congestion of lungs which cleared up within eight days. 14 hours' exposure caused death within twenty-four hours	Very irritating to eyes and nasal passages. Deep inhalations nauseating. Intolerable atmosphere.

It should be noted that 6 mg. l. is the highest concentration of dichloroethyl ether in air which can be reached at ordinary temperatures. Concentrations approaching saturation are highly toxic, but the warning properties are so good that no one would voluntarily remain in an atmosphere with a dangerous concentration for long enough to suffer harm. In this respect dichloroethyl ether is preferable to methyl bromide, which has no smell and produces no immediate symptoms when dangerous amounts have been inhaled.

*Experimental.*—By using the apparatus and technique already described, the toxicity of dichloroethyl ether vapour at different concentrations was tested against the cheese-mite, *Tyrolichus casei* Ouds. The required quantity of dichloroethyl ether was measured out in liquid form by means of a micropipette.\* One division of the pipette corresponded to 8.61 mg. of dichloroethyl ether, which, when vaporized in the apparatus, which had a capacity of 134 l., would produce a concentration of 0.064 mg. l.

\* We wish to thank Dr. Askew for assistance in this connection.

The results are recorded in two separate tables. Table XII shows results of exposure of mites to the lowest concentrations of dichloroethyl ether used, but with variations in the temperature and time factor. Table XIII is a record of results of treatment of mites at different concentrations of dichloroethyl ether, but with the time and temperature factors kept constant. Relative humidity in both tables was in the vicinity of 77 per cent. The mortality amongst the untreated mites was dealt with in the same way as in the methyl bromide experiments.

TABLE XII.—TABLE SHOWING EFFECT OF LOW CONCENTRATIONS OF DICHLOROETHYL ETHER ON MITES

Dichloroethyl ether concentration : 0.004 mg./l. = 0.004 lb. per 1,000 cubic feet.  
Relative humidity : 77 per cent.  $\pm$  5 per cent.

Experiment No.	Number of Mites used.	Percentage Mortality.		Time exposure (Hours).	Temperature (° F.).
		Untreated.	Treated.		
1 .. ..	165	2.5	88.2	65	68
2 .. ..	191	10.5	91.5	72	68
3 .. ..	181	7.5	54.2	72	60
4 .. ..	160	11.8	48.9	72	69

The results in this table indicate that only a trace of dichloroethyl ether is necessary to control the mites when a thorough distribution of its vapour can be obtained and its concentration maintained.

TABLE XIII.—TABLE SHOWING EFFECT OF DICHLOROETHYL ETHER AT DIFFERENT CONCENTRATIONS ON MITES WHEN OTHER FACTORS ARE CONSTANT

Relative humidity : 77 per cent.  $\pm$  5 per cent. Temperature : 70° F.  $\pm$  2° F.  
Time of exposure : 4 hours.

Series.	Milligrams per Litre.	Pounds per 1,000 Cubic Feet.	Number of Mites used.	Percentage Mortality.	
				Untreated	Treated.
1 .. ..	0.382	0.024	252	2.5	92
	0.382	0.024	246	5.4	82
	0.318	0.020	200	2.7	65
	0.446	0.028	214	2.7	75.5
	0.255*	0.016	182	2.7	38
2 .. ..	0.382	0.024	272	5.2	88.3
	0.446	0.028	220	0	94.5
	0.509	0.032	242	5.5	96
	0.573	0.036	232	20	97
3 .. ..	0.382	0.024	201	0	4
	0.446	0.028	197	0	48.8
	0.509	0.032	209	0	52.5
	0.573	0.036	206	0	86
4 .. ..	0.382	0.024	196	6.2	80.6
	0.446	0.028	189	5.2	71
	0.509	0.032	181	10.2	92
	0.637	0.040	177	2.5	96.6

\* Not taken into the calculations, since the temperature at which this experiment was carried out was 73.8° F.

The experiments in series 2 and 3 of Table XIII are each strictly comparable, one with the other—that is to say, that each experiment at the given concentration in both series was conducted at the same time and thus exposed to the same set of conditions. The difference between the series was the environment from which the mites were taken. The mites for series 2 experiments were taken from cheese exposed to daily fluctuations of summer temperature and humidity, while the mites used for series 3 experiments were taken from cheese held in cold store at a constant temperature of approximately 45° F. and fluctuating humidity. Why mites from one environment proved more susceptible to a toxic agent than mites from another environment remains unexplained. The idea might be advanced that metabolic processes of the mites from the cold environment may be considerably slowed down as compared with that of those from a warm environment, with the result that reaction to the fumigant in the former case is much slower than it is in the latter. Again, in the table it will be noted that different mortality results occur in using the same concentration of gas, but this is probably explained by unevenness in the samples themselves.

The response of the mites to the small differences in concentration of the dichloroethyl ether is worthy of note and is best illustrated in series 3 experiments, where successive increases of dosage of the order of 0.064 mg. l. show a corresponding increase in percentage mortality.

Dosage mortality curves were calculated from the data shown. Because of the differences of environment from which the mites used in series 1, 2, and 4 were taken as compared with those used in series 3, the experiments in the 1, 2, and 4 series were treated statistically as experiments in one series and the experiments in series 3 as those in another. The results are given in Table XIV, which follows:—

TABLE XIV

Series.	Values for $\chi^2$ .			Difference in $b$ the Slope of the Line			
	$n$ .	$\chi^2$ .	P.	$b$	Variance of $b$ .	Pooled Variance	$t$ .
1, 2, 4 .. ..	4	49.934	0.05	4.167	2.6	6.7	1.696
3 .. ..	2	37.848	0.05	13.532	15.5	..	..

The large  $\chi^2$  indicates that there has been some uncontrollable factor in the experiment, thus causing large fluctuations in the numbers killed for each dosage. This perhaps is not surprising considering the very small dosages of dichloroethyl ether that are required to bring about a relatively large increase in the percentage of mites killed. Very small fluctuations of dosages as measured by a micropipette, or differences in relative ages of the mites used in the experiments, might easily be reflected in the final mortality counts. In Fig. 5 the regression lines and their zones of error have been calculated, the latter having been plotted as broken lines from the data given.

*Effect of Dichloroethyl Ether on Cheese.*—A loaf cheese was placed under a bell-jar with a petri dish containing dichloroethyl ether. On examining the cheese four weeks later the outside was found to have a strong odour of dichloroethyl ether, but the inside was free from taint.



One loaf cheese (unwaxed) and one 40 lb. medium cheese (waxed) were sprayed all over with dichloroethyl ether at the rate of 2.5 g. per square foot of surface area (= 0.5 lb. per 100 square feet). Cheeses were sampled after exposure to air for one week. In each case the interior was free from taint. The rind of the loaf cheese had a distinct smell of dichloroethyl ether, but no smell could be detected on the exterior of the medium cheese. The odour on the exterior of the loaf cheese gradually became less pronounced and could not be detected after a further one or two weeks.

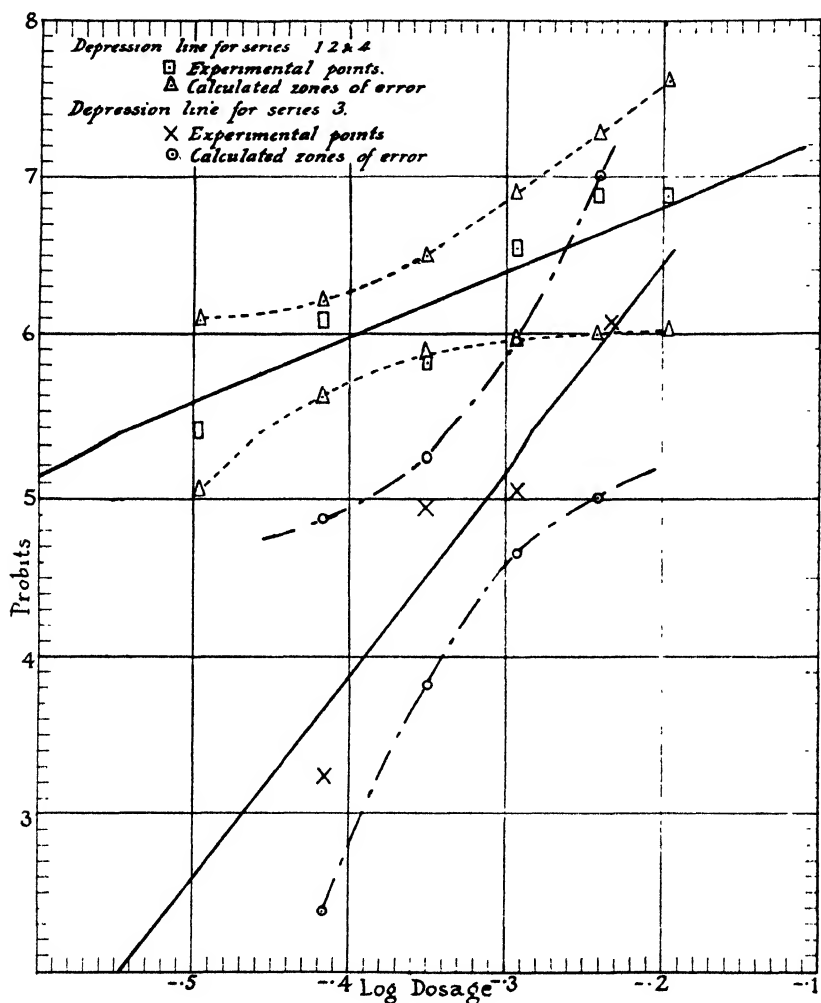


FIG. 5

*Estimation.*—The estimation of dichloroethyl ether in air presented considerable difficulties, as the concentration used in fumigation was very low. Dichloroethyl ether in air has been determined by Schrenk, Patty, and Yant(17) by aspirating a measured volume of air through two bubbling tubes containing saturated alcoholic potash. The contents of the tubes were then refluxed for one to two hours and chloride was determined by

Volhard titration. Trials of this method did not give satisfactory decomposition of the dichloroethyl ether, but reduction with sodium and alcohol according to the Stepanov method gave recoveries of close to 100 per cent. with weighed quantities of dichloroethyl ether. The method adopted was as follows: a measured volume of air (1 l. to 4 l.) was aspirated slowly through two potash bulbs connected in series. Each bulb contained about 7 ml. of alcohol. When the required volume of air had been passed (ten to thirty minutes), the bulbs were emptied and rinsed out with alcohol. Chloride in the alcohol solution from each bulb was determined separately by Volhard titration following reduction with metallic sodium. Usually at least two-thirds to three-quarters of the chloride absorbed was found in the first bulb, indicating that the two bulbs together should have given a fairly complete absorption.

*Applied Experiments.*—It will be realized that the laboratory experiments already described were conducted under exact conditions, and one cannot hope to achieve the same degree of success in destruction of mites when applied in a practical way. Many difficulties arise in the practical application—e.g., temperature and humidity ranges are not controlled, there may be difficulty in rendering the room gastight, and there will be loss of dichloroethyl ether by absorption by the different substances within the room. Having in view many of the above difficulties, the following practical experiments using different methods of approach were carried out.

In a small room of 1,030 cubic feet capacity four heavily mite-infested cheeses were fumigated with 0.097 lb. of dichloroethyl ether—this quantity when fully vaporized would give a concentration of 1.5 mg. l. The dichloroethyl ether was vaporized from blotting-paper, wrapped around a heated (150 F.) metal surface over which a current of air was passed by means of a small fan. The temperature of the room was 48 F. and the relative humidity 68 per cent. The experiments continued for six hours, after which time the room was aired and samples of mites were collected from different positions and mortality counts made. At the commencement the four cheeses were placed on flat boards on a table. One cheese was wedged up slightly so as to permit the fumigant to permeate the space between the base of the cheese and the board on which it rested: a sample of mites was also placed near the cheeses in open petri dishes. Three control samples were taken, two from underneath the wedged cheese and one from the top of another cheese. The results are shown in Table XV.

TABLE XV

Position Mites taken from	Number Dead	Number Alive
From base of treated wedged cheese .. ..	0	20
From base of treated wedged cheese .. ..	1	19
From top of treated wedged cheese .. ..	1	23
From top of treated wedged cheese .. ..	2	18
From open petri dishes .. ..	8	13
From open petri dishes .. ..	8	15
From control (untreated) .. ..	0	20
From control (untreated) .. ..	1	19
From control (untreated) .. ..	1	19

The results in Table XV indicate that the degree of control obtained was negligible where the mites were treated in their normal environment, but away from this environment, as in the open petri dishes, a noticeable mortality resulted. The experiment was repeated, but the quantity of dichloroethyl ether used was doubled, giving a dosage of 0.194 lb. per 1,000 cubic feet of space or 3 mg./l. when fully vaporized. The room was not aired until twenty-four hours after the experiment commenced. The results are given in Table XVI.

TABLE XVI

Cell No.	Position Mites taken from	Number Dead.	Number Alive.
K	From petri dish 6½ hours after experiment commenced ..	28	0
4	From cavity in top of cheese .. .. .	25	0
J	Scraped from top of same cheese as 4 .. .. .	32	0
8	From top of same cheese as 4, but less exposed ..	25	1
14	From rind near base of cheese, but not underneath ..	25	0
M	From base of cheese resting flat on board .. ..	3	19
6	From base of wedged cheese .. .. .	40	0
Z	From base of wedged cheese as cell 6, but more protected position	6	13
W	Control .. .. .	0	20
C	Control .. .. .	2	18

Table XVI shows that a dosage of 0.194 lb. of dichloroethyl ether per 1,000 cubic feet of space—five to six times greater than the highest experimental concentrations used—is sufficient to kill all mites in an exposed position, but that penetration is poor, with the result that mites underneath a cheese, for example, are hardly affected.

*Application of Dichloroethyl Ether as Vapour to a Vell-storage Room.*

A trial fumigation with dichloroethyl ether applied as vapour was made in a cool room used for storing vells which had become infested with mites (*Glycyphagus domesticus* de Geer). The vells, which were packed close together, were tied in bundles and then placed in four bushel sacks. The sacks were stacked on both sides of the vell-room, leaving a clear passage down its centre. This room, which was 14,000 cubic feet in capacity, was normally maintained at about 40° F. It had no openings except for two doors of the cold-room type. A system of ducts connected with a housing outside the room containing a fan and cooling coils provided for air circulation and temperature control. The temperature was allowed to rise to approximately 50° F. before commencing the fumigation. The vaporizing apparatus (Fig. 5) consisted of a strong metal stand to which was riveted a small tank, which held approximately 6 gallons of liquid. Vaporization was assisted by three factors: application of heat, use of a large evaporation surface, and rapid removal of the vapour as it formed. Heat was obtained from two thermostatically controlled immersion heaters—the temperature was not allowed to rise above 170° F.—the evaporation surface consisted of fifteen copper plates each of which was covered with absorbent cloth, and the vapour formed was removed by means of an electrically driven fan. In operation the heated dichloroethyl ether was soaked up by the cloth on to the large evaporation surface, and the vapour, as it formed, was removed by means of the fan, which forced the air across the evaporation surfaces and out into the room.

The vaporizer was run for three hours, during which 6 lb. of dichloroethyl ether was evaporated. The large fan was run to assist in circulating the vapour. A considerable amount of leakage was noticed from the fan housing outside the room, and at the end of the run the concentration of vapour in the room was still apparently quite low, as it caused no discomfort on entering the room. This assumption was confirmed by analyses of samples of air drawn from the fan duct, as shown in Table XVII following :—

TABLE XVII

	Milligrams per Litre as D.C.E.
Dosage delivered .. .. .	6.0
Blank run before fumigation started .. .. .	0.4
End of vaporization .. .. .	0.3
One to one and a half hours after end of vaporization .. .. .	0.9
Four and a half hours after end of vaporization .. .. .	0.2
Eighteen hours after end of vaporization .. .. .	0.4

The blank run showed that there was a considerable amount of chloride present in the air before any dichloroethyl ether was vaporized. As the amount was probably not constant, the accuracy of the determination would be reduced accordingly. It is clear, however, that the vapour concentration produced throughout the room was very small. A large proportion of mites in specimen tubes survived.

A further fumigation was carried out on the following day. The external fan was not run except when taking samples, so that leakage from its housing was largely eliminated. The vaporizer was run for six hours during which 8½ lb. of dichloroethyl ether was evaporated. The dosage was thus 0.6 lb. per 1,000 cubic feet, or 9 mg. l. Analyses of air samples are given in Table XVIII following :—

TABLE XVIII

	Concentration found (mg. l.).	Concentration corr'd (mg. l.).
Dosage delivered .. .. .	9.0	..
End of vaporization .. .. .	3.5	3.1
Seventeen hours later .. .. .	1.8	1.4

According to these results, an effective concentration had been maintained for a considerable period. A 100 per cent. kill of mites in sample tubes in various parts of the room was obtained. Most of the bags of vells were not obviously infested with mites before fumigation. At a later date one bag with living mites was found, indicating that penetration had not been complete.

This method has the advantage that it can be controlled from outside the room and is also a satisfactory means of treating a store filled with cheese. It cannot be applied in a room the construction of which makes effective sealing impossible, as leakage losses may prevent an effective concentration being reached. The amount of liquid vaporized should be approximately 8 oz. for each 1,000 cubic feet of space in the room, to allow for losses by leakage and absorption. Cheese on the shelves should preferably be wedged up to allow vapour to penetrate underneath. After keeping the room closed for twenty-four hours, it can be opened and aired.

*Cheese-curing-room Trials.*—Two methods were experimented with: in the first, dichloroethyl ether was applied directly to the shelving, while in the second it was applied to scale boards, which were later placed under the cheeses.

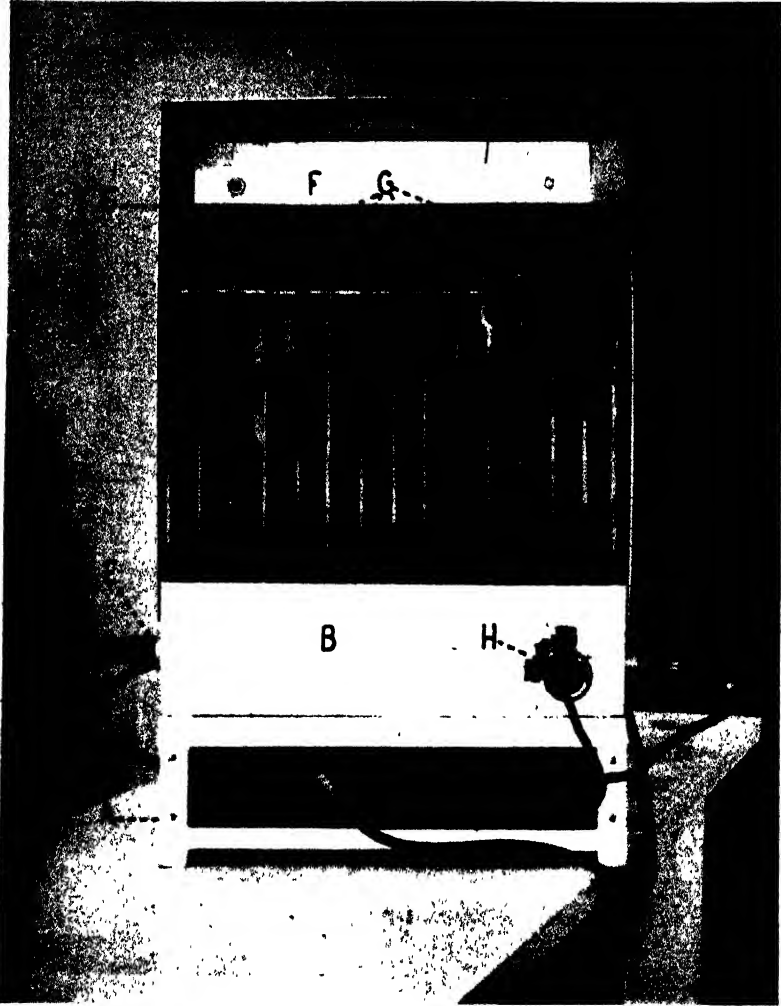


FIG. 6A

Figs. 6A and 6B.—Front and side views respectively of vaporizing-apparatus. A, rigid welded metal stand, 16 in.  $\times$  24 in.  $\times$  5 in.; B, tank, 16 in.  $\times$  13 in.  $\times$  4 in., made from thin sheet metal; C, hood, 16 in.  $\times$  17 in.  $\times$  13 in., made from thin sheet metal; D, motor and fan; E, heating coil plugs; F, baffle (placed so as to give draught from fan downward tendency); G, copper plates,  $\frac{1}{8}$  in.  $\times$  12 in.  $\times$  12 in. (may be covered with cloth or any material which readily absorbs dichloroethyl ether).

(a) *Treatment of Shelving:* The first trial of this method was made in a small cheese-curing room at the Dairy Research Institute. This room was 10 ft. square and 10 ft. high. Temperature was automatically controlled at 45° F. and relative humidity at approximately 85 per cent. An infestation of mites had been present for some two or three months. The cheeses were

removed from the room and a total quantity of 1.2 lb. of dichloroethyl ether was applied to the shelves. The area of shelving was 180 square feet, so that the rate of application was 0.66 lb. per 100 square feet. Application to most of the shelves was made by spraying, but a few were treated with a 10 per cent. emulsion of dichloroethyl ether in water. It was found, however, that the volume for each shelf was such that the liquid tended to run off on to the floor. Where undiluted dichloroethyl ether was sprayed on at the same rate (0.66 lb. per 100 square feet), the quantity was just

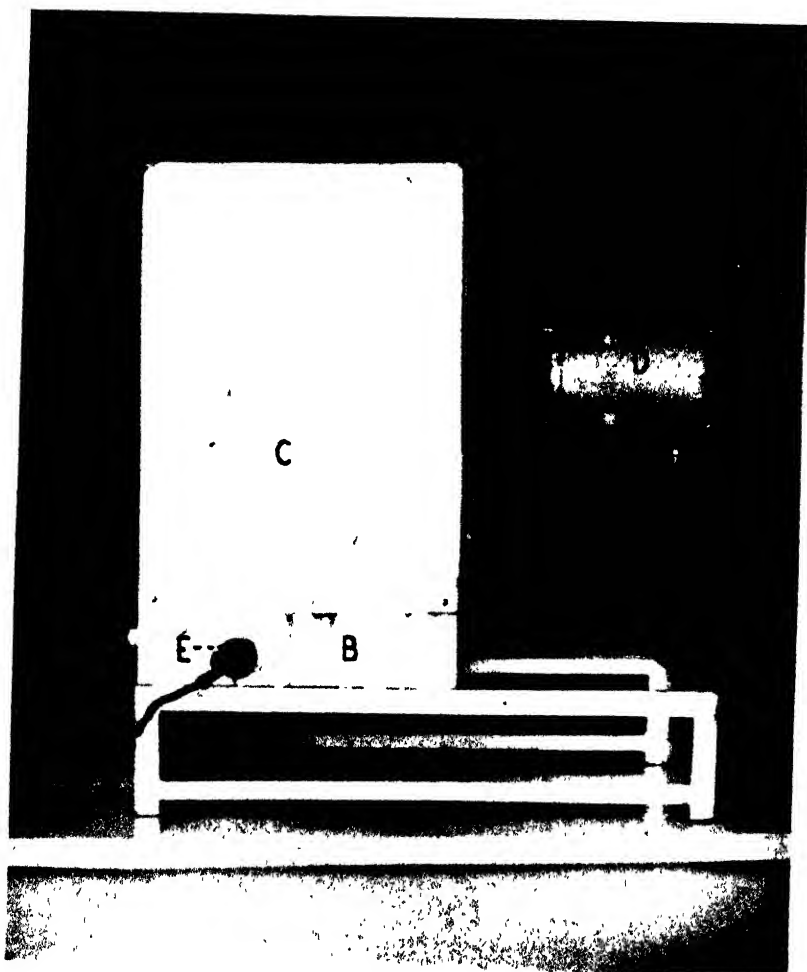


FIG. 6a

sufficient to wet all the surface of the wood. The room was kept closed overnight, and was opened next morning just long enough to replace the infested cheeses. The concentration of vapour in the air at this time was high and it was impossible to remain more than a few seconds in the room if a gas-mask was not worn. After three days the room was opened and aired and the cheeses examined. It was found that all the mites had been killed.

The odour of dichloroethyl ether was noticeable in the room for some weeks, but was not objectionable. The room remained free from mites for eight months, when the introduction of mature cheeses from another store brought about a fresh infestation.

A further trial was made in the curing-room of a commercial factory in which a heavy infestation of mites was present. The room measured 30 ft.  $\times$  28 ft.  $\times$  12 ft., giving a cubic content of 10,080 cubic feet, and contained 1,020 square feet of shelving, sufficient for about five hundred cheeses. The room had plastered brick walls, concrete floor, and a wooden ceiling. The construction was such that the room could be closed up fairly completely to prevent escape of vapour. Dichloroethyl ether was applied to the shelves with a paint-brush, while old crates and other materials in the room and portions of the floor were lightly sprayed. Application by brush was found to require more liquid per square foot than spraying, but was more convenient than a hand-operated spray-syringe. A gas-mask was worn throughout the operations. The total quantity of dichloroethyl ether used was 14 lb. The cheeses in the store were moved to allow application of the liquid to the shelves, and were replaced as soon as the treatment was finished.

A determination of dichloroethyl ether in the air in the centre of the room about one hour and a half after the treatment was completed gave a concentration of 0.7 mg./l. Examination of the cheeses three days later showed that a 100 per cent. kill of mites had been obtained. The cost of dichloroethyl ether used for this treatment was £1 16s.

The results obtained indicated that this should be a convenient and effective method of treating a cheese-curing room, provided that the treatment could be applied at a time when the store was only partly filled with cheese. The evaporation of liquid from the shelves soon builds up a sufficiently high concentration of vapour in the air to fumigate the whole room, while traces of dichloroethyl ether remaining in the wood prevent reinfestation by mites on the shelves or under the cheeses for a considerable time. In order to allow an ample margin for losses of vapour by leakage or diffusion, the rate of application should be of the order of 1 lb. per 1,000 cubic feet of room space. In an average curing-room this will correspond to about 1 lb. for shelving for one hundred export cheeses or 1 gallon for shelving for one thousand two hundred cheeses. Where application is made by brushing, a rather larger quantity will be needed to cover all the surface of the shelving. The operator applying the liquid must, of course, wear a gas-mask. After the empty shelves have been treated, it is necessary to move the cheeses in the room on to the treated shelves so that the remaining shelves can be treated. Handling of cheeses can be kept to a minimum by arranging that cheeses need only be moved to an adjacent shelf on the same level. After the treatment, the room should be kept closed for a day or two, after which it may be ventilated.

(b) Use of Treated Scale Boards: The following three methods were used for treating scale boards with dichloroethyl ether:—

- (1) The edges of scale boards were dipped in a tray of dichloroethyl ether to a depth of approximately  $\frac{1}{2}$  in. and allowed to drain. Sixty scale boards were treated in this manner. Weights taken before and after dipping showed that 14 oz. of the liquid had been taken up. The boards were placed under the cheeses, and an examination four days later showed that mites were all killed in the vicinity of the dichloroethyl ether, but in positions remote from it—that is, near the centre of the base of the cheese—live mites could still be found. Fourteen days later there was no change.

- (2) The edges of the scale boards were dipped to a depth of approximately 2 in. and allowed to drain—a hole was bored at the centre of these boards and then filled with  $1\frac{1}{2}$  oz. of dichloroethyl ether. Sixty scale boards were treated, with the result that  $31\frac{1}{2}$  oz. of dichloroethyl ether were taken up. The boards were placed under cheeses as in the previous experiment. After four days an examination showed that all mites on the base of the cheese had been killed and also many had been killed at a slight distance away from the outside rim of the cheese. Fourteen days later there was no change.
- (3) The scale boards were first soaked in water, and then put through a wringer to squeeze out excess moisture. The boards were then submerged in a dish of dichloroethyl ether and allowed to drain. Sixty boards were treated in this fashion, with the result that 12 oz. of dichloroethyl ether were taken up. The boards were placed under the cheeses as before, and on examination four days later live mites were still present. Fourteen days later all mites at the bases of the cheeses were dead.

If scale boards are completely immersed in dichloroethyl ether, the amount absorbed is more than is required to give effective control of the mites, so that some method such as (2) or (3) can be used to limit the amount taken up while at the same time giving a fairly uniform distribution of dichloroethyl ether over most of the surface of the scale board. The results secured from methods (2) and (3) suggest that use of treated scale boards would be of value in a store which is well filled with cheeses. If, in addition to placing scale boards under the cheeses, a treated scale board is laid on top of each cheese, an effective fumigation should be secured.

*Discussion.* Dichloroethyl ether applied as a vapour required special vaporizing-apparatus and a gastight room. Penetration by the vapour is poor, and in this respect it is much inferior to methyl bromide. Application to shelving is simple and effective, but requires the operator to work in a gas-mask. It is difficult to apply when the shelves are full of cheese, but in such circumstances the placing of a treated scale board beneath the cheese provides a practical alternative method. The scale-board method, however, will not give a complete kill on the upper part of the cheese, especially if the room be draughty, but this drawback may be overcome by placing a treated scale board on top of the cheese as well as beneath it.

### GENERAL CONCLUSIONS

It has been shown that dusts are not effective in preventing infestation of cheese by mites, and that the wax-coating of cheese, though delaying mite attack to some extent, does not provide a permanent protection. As far as prevention of damage by mites is concerned, there is probably some advantage in waxing cheeses which are to be stored for a long period, but the only really satisfactory method is by periodic fumigation of the curing-room combined with measures to reduce the amount of reinfestation.

Of the two fumigants with which successful results were obtained, methyl bromide does not appear to be very suitable for use in factory curing-rooms, mainly on account of cost, but it might have a useful application where it can be used in a confined space such as a fumigation chamber—*e.g.*, for



treating mature cheese coming into a store. Its remarkable penetrative power would be valuable in the fumigation of crated cheese. Dichloroethyl ether should provide a cheap and effective means of keeping factory curing-rooms free from mites, and if applied to the shelving or on scale boards as described should be sufficiently lasting in its effect to make an annual treatment sufficient.

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## CONTROL OF MOISTURE CONTENT OF BUTTER DURING BUTTER MANUFACTURE

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### Summary

The straight percentage method of calculation of quantity of make-up water to be added to partially worked butter in a churn to bring the moisture content to approximately the legal limit is incorrect, because the first moisture test is based on the unfinished butter and the final test on the finished butter. The correct calculation is most conveniently made by the formula—

$$\text{Quantity to be added} = \frac{\text{desired moisture content (\%)}}{100 - \text{actual moisture content (\%)}} - \frac{\text{actual moisture content (\%)}}{\text{estimated load of butter in churn}}$$

A chart calculated on this basis is presented. The chart is not applicable for calculation of quantity of salt to be added.

THE legal limit for moisture content of butter, both in New Zealand and Great Britain, is 16.0 per cent., and it is an advantage to the manufacturer from the economic point of view to bring up the moisture content as nearly as possible to the limit. The despatch from the factory of butter with moisture content over 16.0 per cent., on the other hand, involves the factory in heavy expenditure for freight, wrappings, containers, and labour, as the butter is returned from the grading-store at the factory's expense and must be reworked with fresh butter. In controlling the moisture content of butter, therefore, it is the duty of the buttermaker to raise the moisture content as nearly as possible to 16.0 per cent. without overstepping the limit. On account of variation in moisture content in the samples taken for analysis, both at the factory and at the grading-store, and of the slight possible error in testing, it is not generally considered safe to aim at an average moisture content higher than 15.80 per cent., although at some factories the average for the year exceeds this value.

The control is effected as follows:—

After working the butter, the buttermaker observes the flow of chill-water from the granules through the churn-plugs until in his judgment there is just sufficient water left in the churn to give, when worked into the butter, a final moisture test above 15.0 per cent., but below 16.0. The churn-plugs are then shut, the salt is added on top of the granules, the churn is rotated a short time to give thorough mixing-in of the salt, and the butter is worked until the churn is dry. There should be no appreciable quantity of free water on a trier plug taken from the butter in the churn. A sample is then taken for the first moisture content. Based on the result of the test, a quantity of additional water is added to bring the moisture content up to the desired value, and the working is continued until the added water is worked into the butter and the butter has the correct body.

## CONDITIONS AFFECTING ESTIMATE OF AMOUNT OF WATER TO BE ADDED

(1) *Estimation of Load of Butter in Churn.*—Accurate assessment of the quantity of water to be added is possible only if the load of butter in the churn is accurately known. The method commonly recommended in text-books on buttermaking is to measure the volume of cream placed in the churn, and to estimate the fat content of the cream by the Babcock or Gerber method. It is then necessary to calculate the expected yield of butter from the churn on an assumed churn overrun. Hunziker(1) assumes a churn overrun of 25 per cent. In the Manual for Dairy Manufacturing Courses of the Pennsylvania State College(2) a churn overrun of 23 per cent. is assumed. These overruns apply under United States conditions, where the regulations require only that the butter shall contain 80 per cent. of fat. Turner and Graham(3) assume a churn overrun of 21.9 per cent. Any assumed overrun will give consistent results only so long as the percentage loss of butterfat in the buttermilk is constant from churning to churning, and the final moisture and salt contents are uniform. Butterfat loss is influenced by temperature of churning, degree of dilution of cream, and by many other factors. Hence, even with an accurate knowledge of the volume and fat test of the cream in the churn, there is a degree of uncertainty as to the expected yield of butter.

In New Zealand factories weighing of the cream as it is drawn from the 3,000-gallon holding-vats to the churn is not possible, and the buttermaker is usually too busy with the churning to carry out fat tests on the cream. The common practice is to *estimate* the load of finished butter in the churn in terms of 56 lb. boxes. Some buttermakers take particular care to fill the churn always exactly to the same mark with cream or to measure the quantity drawn from the holding-vat by means of a calibrated measuring-rod on the assumption that the average fat content of cream at the factory remains constant. This procedure is of some assistance, but may lead to serious mistakes if relied upon as the sole index. There may be appreciable variations in amount of froth on the cream in the churn or the holding-vat and variation in degree of dilution of the cream during processing, and also there is always some further dilution in some churnings from water used for rinsing out the holding-vats. The more usual method is to judge the churn load of finished butter from the volume of granules in the churn, with due allowance for any variations in granule size and in softness of the butter. A good buttermaker, after some experience with his churns, can judge the load fairly regularly to within two to three boxes in a load of eighty to ninety boxes.

An error in the estimation of churn load is of more importance when the first moisture test is low than when the first moisture test is near to the limit. In the calculation of amount of water to be added a margin is usually allowed to cover the various errors—viz., estimation of load of butter, errors in sampling, and errors in moisture-testing. When the first moisture test is high this margin provides proportionately a larger safety reserve to cover error of estimation of churn load than if the first moisture test is low—e.g., if the first moisture test is 15.3 and the final result aimed at is 15.8, the safety margin of 0.2 is 40 per cent. of the desired increase

in moisture content. If, on the other hand, the first moisture test is 13.0, the safety margin of 0.2 is only  $\frac{0.2}{2.8} \times \frac{100}{1} = 7$  per cent. of the desired increase in moisture content. A 7 per cent. error in the estimate of churn load (seven boxes in 100 or three boxes in 40) would then be the maximum error permissible even if no other possible error were to operate in the same direction. With the churning giving a high first moisture test, on the other hand, considerably more latitude in the degree of accuracy of estimation of churn load is permissible. For this reason it is the practice of some buttermakers to add the water in two stages when the first moisture test is low. This is possible only when the butter will stand the extra working without becoming too sticky.

The first moisture test is affected primarily by the degree of draining of the granules allowed before the churn-plugs are closed. The butter-maker judges the time to close the churn-plugs on the appearance of the stream of water flowing from the chute, and uses the previous day's results to guide him. The draining of the granules is affected however by —

- (a) *Size of Grain*: Large granules drain more completely than small granules:
- (b) *Temperature of Butter*: Soft granules retain moisture more readily than hard granules.
- (c) *Fat Content of Cream at Churning*: With a churning containing much added water more water will be retained in the granules.
- (d) *System and Intensity of Cream Treatment*: Granules from vacreated cream tend to retain more moisture than granules from flash-pasteurized cream.

(2) *Accuracy of Sampling*.—It is a prime necessity for accurate moisture control that the churn should give a uniform moisture content throughout the butter. When uniformity is not obtained the sample should be taken from the butter in the churn at the place where butter with the highest moisture content is usually found. It is an offence to deliver *any portion* of a churning of butter with moisture content over the legal limit, and the safety margin allowed by the butter-maker must cover the possibility of error in sampling. The modern internal-worker churn gives a fairly uniform moisture distribution, but it must be set perfectly level so that the water does not accumulate at one end during the working.

#### CALCULATION OF AMOUNT OF MAKE-UP WATER TO BE ADDED

The calculation of amount of make-up water to be added has usually been made by the straight percentage method, as in the following example:—

First moisture content	..	..	..	..	15.0 per cent.
Desired moisture content	..	..	..	..	15.8 per cent.
Estimated load of finished butter in churn	..	..	..	..	80 boxes, 4,480 lb.
Water to be added	..	..	..	..	$\frac{15.8 - 15.0}{100} \times 4,480$ $= \frac{0.8}{100} \times 4,480 = 35.8$ lb.

Various charts drawn up on this basis of calculation have been prepared and are in use in butter-factories. The basis of calculation, however, is in error. The first moisture test is taken on the unfinished butter and the second on the finished butter. Before the straight-percentage method could be used it would be necessary to correct the first moisture test to the basis of finished butter. It is simpler to calculate the amount of water to be added by the following formula :—

$$\frac{\text{Desired moisture test} - \text{first moisture test}}{\text{Water-free solids in 100 parts of unfinished butter.}} \times \text{load of butter in churn}$$

*e.g.*, using the figures in the above example—

$$\begin{array}{rcl} & \text{First moisture test} - 15.0 & \\ \text{Water-free solids in 100 parts of unfinished butter} - & 100.0 - 15.0 & 85.0 \\ & \text{Desired moisture content} - 15.8 & \\ \text{Weight of water to be added} - & \frac{15.8 - 15.0}{85.0} & 4,480 \\ & = \frac{.8}{85} \times 4,480 & 42.1 \text{ lb.} \end{array}$$

or 6.3 lb. more than the quantity calculated by the straight-percentage method.

Various methods of making allowance for the error of the charts based on the straight-percentage system of calculation have been used in practice. Many buttermakers have aimed at a final moisture content of 16.0 per cent., but obtained an average of only 15.8 per cent. Others have added the quantity indicated by the chart plus one-sixth or one-seventh this quantity. The latter method gives a very close approximation to the correct quantity required. (With these allowances indicated by experience a very satisfactory degree of moisture content has been obtained in practice, as shown by the average moisture content of the butter reaching the Auckland stores—viz., 15.67.)

#### DERIVATION OF FORMULA

The formula may be derived as follows: let W = weight of water to be added to 100 parts of unfinished butter of 15.0 per cent. moisture content to give a finished butter of 15.8 per cent. moisture content.

$$\begin{aligned} \text{The final percentage moisture content} &= \frac{15 + W}{100 + W} \times 100 = 15.8 \\ 1,500 + 100 W &= 1,580 + 15.8 W \\ 100 W - 15.8 W &= 1,580 - 1,500 \\ 84.2 W &= 80 \\ W &= \frac{80}{84.2} \\ \text{Weight of finished butter from} &= 100 + W = 100 + \frac{80}{84.2} \\ \text{100 parts of unfinished butter} & \\ \text{Hence water to be added (W) expressed} &= \frac{W}{100 + \frac{80}{84.2}} = \frac{80}{100 + \frac{80}{84.2}} \\ \text{as fraction of the finished butter} &= \frac{80}{8,420 + 80} = \frac{80}{8,500} = \frac{0.8}{85} \end{aligned}$$

#### BUTTER MOISTURE CONTROL CHART

A chart calculated on the correct basis is given in Table I.

TABLE I.—WEIGHT OF WATER (POUNDS) TO BE ADDED TO BUTTER IN A CHURN FOR DIFFERENT DESIRED INCREASES IN MOISTURE CONTENT OF THE UNFINISHED BUTTER, ON THE BASIS OF A DESIRED MOISTURE CONTENT OF 15.80 IN THE FINISHED BUTTER

Number of 56 lb. Boxes of Butter In Churn.	Desired Increase in Percentage Moisture Content.																			
	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2
10	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.	Bb.
11	2.60	2.65	3.30	4.00	4.60	5.30	5.95	6.60	7.20	7.90	8.50	9.20	9.80	10.5	11.1	11.7	12.4	13.0	13.6	14.3
12	2.50	2.50	3.05	3.65	4.40	5.15	5.90	6.65	7.35	8.10	8.80	9.50	10.2	11.0	11.7	12.5	13.2	13.9	14.6	15.3
13	2.40	2.40	2.90	3.40	4.10	4.85	5.60	6.35	7.10	7.85	8.60	9.35	10.1	10.9	11.7	12.5	13.3	14.0	14.8	15.6
14	2.60	3.45	4.30	5.15	6.00	6.85	7.70	8.55	9.40	10.3	11.1	12.0	12.9	13.8	14.7	15.6	16.5	17.4	18.3	19.2
15	2.80	3.70	4.65	5.55	6.50	7.40	8.30	9.20	10.1	11.0	11.9	12.9	13.8	14.8	15.7	16.7	17.7	18.7	19.7	20.6
16	3.00	3.90	4.85	5.80	6.85	7.90	8.90	9.90	10.8	11.8	12.8	13.8	14.7	15.7	16.6	17.6	18.6	19.6	20.5	21.4
17	3.20	4.25	5.30	6.35	7.40	8.45	9.50	10.5	11.6	12.6	13.6	14.7	15.7	16.7	17.8	18.8	19.8	20.8	21.8	22.8
18	3.40	4.50	5.60	6.75	7.85	8.95	10.1	11.2	12.3	13.4	14.5	15.6	16.7	17.8	18.9	20.0	21.0	22.1	23.2	24.2
19	3.60	4.75	5.90	7.15	8.30	9.50	10.7	11.9	13.0	14.2	15.3	16.5	17.6	18.8	19.9	21.1	22.3	23.4	24.5	25.7
20	3.80	5.05	6.30	7.55	8.80	10.05	11.3	12.5	13.8	15.1	16.4	17.7	18.9	20.2	21.5	22.8	24.1	25.4	26.7	28.0
30	4.00	5.30	6.60	7.90	9.25	10.6	11.9	13.2	14.5	15.8	17.1	18.4	19.7	21.0	22.3	23.6	24.9	26.2	27.5	28.8
40	6.00	7.95	9.90	12.0	14.9	17.8	19.7	21.7	23.6	25.6	27.5	29.4	31.3	33.3	35.2	37.1	39.0	40.9	42.8	44.7
50	8.00	10.6	13.2	16.0	19.1	22.1	25.1	28.0	30.9	33.8	36.7	39.6	42.5	45.4	48.3	51.2	54.1	57.0	59.9	62.8
60	10.00	13.2	16.5	19.8	23.5	27.0	30.5	34.0	37.5	41.0	44.5	48.0	51.5	55.0	58.5	62.0	65.5	69.0	72.5	76.0
70	11.95	15.5	19.85	23.8	28.1	32.4	36.7	41.0	45.3	49.6	53.9	58.2	62.5	66.8	71.1	75.4	79.7	84.0	88.3	92.6
80	13.90	18.5	23.15	27.8	32.7	37.6	42.5	47.4	52.3	57.2	62.1	67.0	71.9	76.8	81.7	86.6	91.5	96.4	101.3	106.2
90	15.90	21.2	26.55	31.7	37.0	42.2	47.4	52.6	57.8	63.0	68.1	73.3	78.4	83.5	88.7	93.8	98.9	104.0	109.0	114.0

Conversion of Fractions of 1 lb. to Ounces

Fractions of 1 lb.	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95
Ounces	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

Additions for churnings containing ten or more than ten boxes of butter can be deduced by direct reading or by addition of two quantities. Additions for less than ten boxes can be deduced from the corresponding value of the multiple of the number by dividing by ten.

The chart is calculated, as stated above, on the basis of a desired average moisture content of 15.80 and, as the result of the operation of other possible sources of error, the final moisture content of individual churnings may vary from 15.60-16.00. If the range of variation of moisture content is found to be lower, the buttermaker may decide to aim at a higher average than 15.80. The figure for "desired increase" in moisture content will then be altered and the chart will still indicate the quantity of water to be added.

#### *Possible Errors in the Use of the Chart*

As stated earlier, even with an accurate basis of computation, the accuracy of the moisture control is dependent on the accuracy of the estimate of load of butter in the churn. Apart from this source of error, there are some further factors which must be taken into account :—

(1) If the churn is not as dry at the first moisture test as it will be at the second moisture test, allowance must be made for the extra moisture that will be taken up by the butter from the churn. When there is a tendency to the production of sticky butter, complete drying up of the churn for the first moisture test may bring the working to such a stage that the butter will have to be overworked to incorporate the added moisture. The buttermaker, for the first moisture test, must then stop the working before the churn is completely dry and use his judgment as to what allowances should be made for moisture retained in the churn. In this respect different allowances must be made according to the individuality of the churn. An old churn in which the wood is spongy will retain more moisture than a new churn.

(2) If working cannot be continued until the churn is dry before the sample for the first moisture test is taken, care is necessary that the sample used for analysis does not contain a droplet of free moisture, and some allowance must be made for possible sampling error.

#### MECHANICAL LOSS OF WATER DURING WORKING

After addition of make-up water the churn-door should be fitted and tightly closed before commencement of working, in order to avoid spillages, which can upset all calculations of amount of water to be added.

#### INFLUENCE OF CLEANNESS OF CHURN ON MOISTURE CONTROL

It is generally accepted that the accuracy of moisture control is greater if the churn can be worked dry before the sample is taken for the first moisture test. Butter sticks to greasy wood much more readily than to clean wood. Hence the cleanliness of the churn has an important relationship to the accuracy of moisture control. A preliminary rinse with warm water to remove the main portion of the residual butter, before the main wash with alkali solution, is an important part of the cleansing routine. A churn will tend to become greasy, causing butter to stick, at the centre of the back end, where washing solutions do not so readily make contact, and on the rollers and the surface of the barrel near the drain-plugs, where the melted fat tends to settle on to the wood during draining of the first rinse water. If trouble is experienced with stickiness at these places in the churn, a rinse with warm water after drainage of the first

wash water may be of some assistance in removing adhering fat. The smaller the amount of butterfat left to be emulsified by the alkaline wash solution, the cleaner will be the churn after final washing with clean hot water.

#### CHART FOR SALTING OF BUTTER

Calculation of amount of salt to be added to butter in a churn is based only on the estimate of the load of finished butter in the churn. The straight-percentage method is therefore valid, and the moisture-control charts which have been in use give a correct indication of the amount of salt to be added. *The chart presented in this paper is not applicable for salt addition.*

#### ACKNOWLEDGMENTS

The author is indebted to Mr. J. J. O'Dea, of this Institute, who first drew his attention to the error of the straight-percentage method in practice, and to a number of butter-factory managers who have tried out the new chart, and reported upon it.

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## THE EFFECTIVENESS OF SMALL APPLICATIONS OF COBALT SULPHATE FOR THE CONTROL OF COBALT DEFICIENCY IN THE SHERRY VALLEY, NELSON

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#### Summary

Wethers have been carried satisfactorily for two seasons on a cobalt-deficient Sherry River pasture after top-dressing with cobalt equivalent of 4 oz. and 8 oz. hydrated cobalt sulphate per acre. The heavier dressing gave the better result both in respect of live-weight increase of the animals and of yield of wool.

A summary of results of animal trials over the last ten years on cobalt-deficient areas in the Nelson District is given.

#### INTRODUCTION

It has been previously reported(1) that at Sherry River, Nelson, two applications of cobalt sulphate at the rate of 2 oz.  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  (equivalent) per acre gave better results than one application at the rate of 4 oz. per acre when sheep were confined on a cobalt-deficient area for two seasons. In continuation of that work the effect of single applications of cobalt sulphate at 4 oz. and 8 oz. per acre in controlling cobalt-deficiency symptoms in wether hoggets over two seasons has been examined.



## EXPERIMENTAL

The area used for the trials has carried sheep for various experiments for three seasons previous to starting the present one. Four blocks each 2 acres in area were available. One of these has received no cobalt applications since experimental work was begun in this district. Those areas to receive cobalt applications were top-dressed, using superphosphate as carrier for the cobalt, on 4th September, 1943, at the rates of 4 oz. and 8 oz.  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  (equivalent). All plots received superphosphate at the rate of 1 cwt. per acre in that season. In the 1944-45 season all plots received superphosphate at the rate of  $1\frac{1}{4}$  cwt. per acre on 7th September, 1944. No further cobalt dressings were given. The fourth plot had been top-dressed on 27th August, 1940, with an experimental cobaltized superphosphate sufficient to provide 16 oz. cobalt sulphate equivalent per acre. No further cobalt dressings had been applied to this area, although in subsequent seasons it regularly received applications of superphosphate.

The history of the various plots since animal trials began on them is as follows:—

Control plot: No cobalt at any period. Kaka ground limestone, 1941-42 season, at 2 tons per acre.

4 oz. cobalt sulphate per acre plot—

Kaka ground limestone, 1940-41 season, at 2 tons per acre.

2 oz. cobalt sulphate equivalent per acre in 1941-42 and 1942-43 seasons.

4 oz. cobalt sulphate equivalent per acre in 1943-44 season, but none in 1944-45 season.

8 oz. cobalt sulphate per acre plot—

Southland limestone, 2 tons per acre, in 1940-41 season.

4 oz. cobalt sulphate equivalent per acre in 1941-42 season, but none in 1942-43.

8 oz. cobalt sulphate equivalent per acre in 1943-44 season, but none in 1944-45.

Cobaltized superphosphate plot: 16 oz. cobalt sulphate equivalent in 1940-41 season, but no further cobalt applications. No ground limestone has been applied to this plot.

Romney cross wether hoggets were sent to the experimental area on 24th November, 1943, and were weighed, tagged, and divided into groups of five sheep of uniform average weight on 25th November. Animals unusually light or extra heavy were omitted from the groups, but were kept at Sherry River on a non-cobalt-treated area for replacement of animals which might die during the period of the trial. The weights of such sheep used as replacements have not been included in the average live-weights recorded in Table I and Fig. 1. By keeping up the number of sheep to five per 2-acre plot, better control of the pasture growth was possible. As far as weather conditions allowed, the sheep were weighed fortnightly and the usual farm attention was given to them.

As shown in Table I, where average live-weights of animals on the several areas are given for selected dates, and in Fig. 1, where a continuous record of average weights is set out, the sheep on all plots at first gained in weight at a satisfactory rate, with no important differences between treatments.

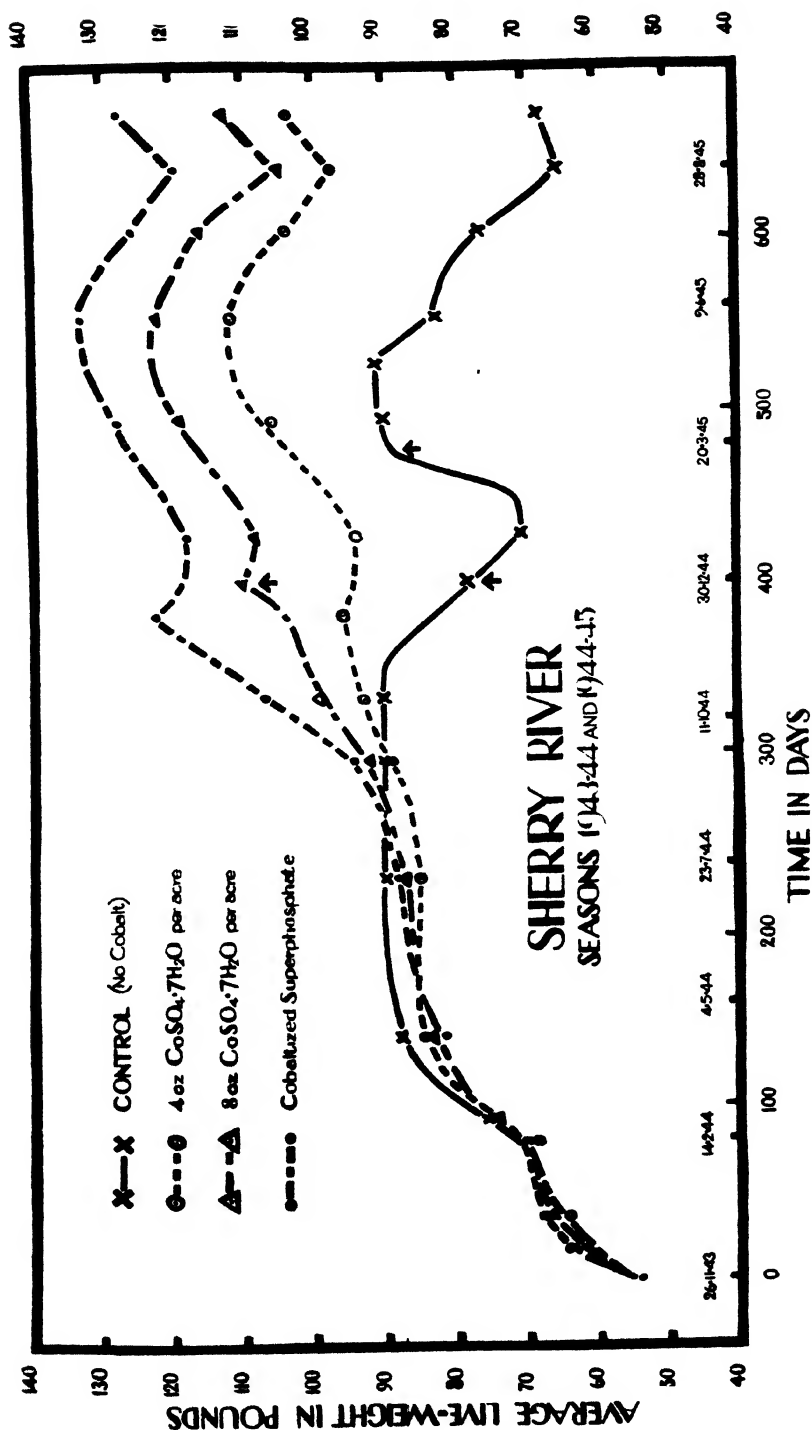


FIG. 1.

After a check to growth towards the end of January, 1944, after about 60 days on the field, steady increases in growth again followed, with the control group showing to some advantage. For a long period, about 142 days to 352 days from the beginning of the trial, the animals on the control area remained very constant in weight at an average of about 90 lb. In the earlier part of this period the animals on the cobalt-treated areas were somewhat below the control group in weight, but nevertheless those receiving cobalt from the 8 oz. cobalt sulphate and cobaltized superphosphate applications were judged to have a more finished appearance with brighter and cleaner wool. Towards the end of this period the sheep on the cobalt-treated areas drew rapidly ahead in weight, the amount of the increase being in the order of increasing amount of cobalt in the fertilizer application. After the end of October, 1944 (about 340 days from commencement of the trial), the sheep on the control (no-cobalt) area began to lose weight rapidly, three of the animals becoming very poor in condition. One of them died during December from cobalt deficiency ("bush-sickness"). This fall in weight in early summer is characteristic of the disease. One sheep was lost from the 8 oz. cobalt sulphate area from an unknown cause, certainly not cobalt deficiency. These losses are shown by the arrows in the curves in Fig. 1.

TABLE I.—AVERAGE LIVE-WEIGHT IN POUNDS OF SHEEP ON TREATED AND UNTREATED PLOTS

Date.	Days.	Control (no Cobalt). (lb.).	4 oz. Cobalt Sulphate per Acre (lb.).	8 oz. Cobalt Sulphate per Acre (lb.).	Cobaltized Superphosphate (lb.)
26/11/43	0	54.0	54.2	54.0	54.0
2/1/44	37	67.6	64.6	62.0	63.3
14/2/44	80	68.8	68.6	68.6	70.2
16/4/44	142	88.0	84.6	83.6	81.6
16/7/44	233	90.0	85.2	88.6	88.2
30/9/44	309	91.6	90.2	94.2	98.6
12/11/44	352	90.0	94.0	101.4	113.4
10/12/44	380	80.6	96.0	103.0	122.6
31/12/44*	401	78.7†	93.6	110.2†	118.4
11/2/45	443	70.7	97.0	110.0	119.8
18/3/45	478	89.3†	105.2	116.5	126.4
22/4/45	512	91.7	111.0	121.2	130.8
3/6/45	554	91.0	111.4	122.0	132.8
24/7/45	605	76.3	103.6	116.0	125.6
29/8/45	641	65.3	97.2	104.7	119.4
29/9/45	672	68.0	103.4	112.2	127.6

\*After shearing.

† Sheep dead or missing since previous weighing.

Another sheep died early in March, 1944, on the control area. No other losses occurred on any of the other treatments after this date. As shown by the weight data, during the summer and autumn of 1945 all groups reached their maximum weights. Thus on 3rd June, 1945 (early winter), 554 days after being placed on the experimental areas, the average weights were as follows: control (no cobalt), 91.0 lb.; 4 oz. cobalt sulphate per acre, 111.4 lb.; 8 oz. cobalt sulphate per acre, 122.0 lb.; cobaltized superphosphate, 132.8 lb. These figures show the great superiority of the animals receiving the cobalt supplement; most of these animals were in fat condition.

During the winter period decreases in weight occurred on all plots, minimum weights being recorded at the end of August, 1945 (611 days). Some increase occurred in early spring at the final record on 29th September (672 days). Owing to continuous wet weather it was not practicable to weigh the sheep before shearing in November. At the end of the trial the sheep were placed in the following order of decreasing health and appearance: 8 oz. cobalt sulphate and cobaltized superphosphate equal, followed by the 4 oz. cobalt sulphate and the control group. In the first two groups the sheep had a good growth of wool and were in fat condition. In the 4 oz. cobalt sulphate group the wool did not appear to be so clean and bright, but the sheep were all fat. The control sheep were in a very poor state. It is evident, therefore, that under the conditions of this trial single applications of both 4 oz. and 8 oz. of cobalt sulphate per acre gave very satisfactory results with the sheep over two seasons, the 8 oz. showing to advantage. It is remarkable that the 16 oz. application of August, 1940, should have continued to give such good results over five seasons, but, as will now be demonstrated, this can be correlated with the relatively high cobalt content of the pasture from this area.

During the progress of the trial pasture samples were taken and analysed for their cobalt content. The results obtained are given in Table II, where the cobalt contents are expressed in parts per million (p.p.m.) of cobalt (Co) on the dry-matter basis.

TABLE II.—COBALT CONTENT OF PASTURE SAMPLES, 1943-44 AND 1944-45 SEASONS

Date of Sampling	Control (no Cobalt) (p.p.m.)	4 oz. Cobalt Sulphate per Acre (p.p.m.)	8 oz. Cobalt Sulphate per Acre (p.p.m.)	Cobaltized Superphosphate (p.p.m.)
27 10 43 ..	0.03	0.22	0.19	0.14
25 11 43 ..	0.05	0.07	0.16	0.11
18 1 44 ..	0.05	0.08	0.11	0.17
29 2 44 ..	0.04	0.08	0.08	0.16
26 4 44 ..	0.03	0.07	0.08	0.19
17 11 44 ..	0.03	0.06	0.12	0.12
8 12 44 ..	0.03	0.06	0.08	0.09
25 1 45 ..	0.05	0.11	0.09	0.18
2 3 45 ..	0.03	0.06	0.06	0.12
27 3 45 ..	0.07	0.09	0.12	0.11

The above data show that the pasture of the control area was consistently low in cobalt, while the pastures on the cobalt-treated areas contained appreciably more cobalt, thus correlating positively with the better health of the stock grazed on them.

During the experiment the sheep were shorn twice, in December, 1944, and on 14th November, 1945. The average weights in pounds of raw wool from the various treatments were as follows:—

Season.	Control (lb.).	4 oz. Cobalt Sulphate per Acre (lb.)	8 oz. Cobalt Sulphate per Acre (lb.).	Cobaltized Superphosphate (lb.)
First .. ..	7.3	7.5	7.7	9.5
Second .. ..	7.0	7.9	8.7	9.7

In the first season the cobaltized superphosphate treatment is outstanding in the amount of wool produced; on the other cobalt-treated areas the increases in yield over the control are almost certainly not significant. This result is not surprising, because the sheep were on the experimental fields for such a short time before they were shorn. Supplementing the cobalt content of the pasture with cobalt sulphate has produced appreciable increases in yield of wool in the second season. These increases in yield are parallel with the increases in live-weight of the sheep on the respective areas.

It may be noted in passing that the deficiency of cobalt at Sherry River appears to be a simple one not complicated by an accompanying deficiency of copper. Thus in samples of pasture taken from the various plots on 6th November, 1945, the copper contents ranged from 9.1 to 11.8 parts per million of Cu on the dry basis. These values suggest that the copper content of the Sherry River pasture was ample for health of sheep.

### DISCUSSION

This paper reports the last of a series of investigations on the effect of cobalt supplements on the health of sheep carried out by the Cawthron Institute on cobalt-deficient areas. Because it is not proposed at present to continue these investigations further, it will be convenient to summarize the investigations, particularly to show how ideas in regard to the quantity of cobalt compounds required to remedy the deficiency have varied since "bush-sickness" was recognized in 1935 as a disease due to a deficiency of cobalt in the pasture grazed by animals.

The first experiments, set up in February, 1936, to supply cobalt to pasture plants were carried out under pot-trial conditions, using hydrated cobalt chloride at the rate of 200 lb. per acre(2). This dressing gave, with crested dogtail, a maximum cobalt content of 122 p.p.m. These were followed by field trials in August, 1936, at the Marsden Research Farm using the same cobalt salt at 112 lb., 20 lb., and 10 lb. per acre. The higher rates caused some damage to the pasture, but the 10 lb. application was quite satisfactory. It was noted in this trial that white clover absorbed more cobalt than did the grasses—for example, for a mixed pasture sample showing 25.2 p.p.m. on the dry basis, the grasses showed 19.1 p.p.m. and the clovers 36.3 p.p.m. when separated out from the sample. No stock were grazed on these small replicated areas. Towards the end of 1936, 2 lb. of hydrated cobalt chloride per acre was applied to a bush-sick pasture at Glenhope, using 2 cwt. per acre of superphosphate as a distributing agent(3). This quantity was sufficient to maintain the cobalt content of the pasture at 0.13 p.p.m. Co on the dry basis as late as five months after application, the cobalt content of the control untreated pasture (receiving phosphate only) being approximately 0.06 p.p.m. "Bush-sick" sheep put on to this cobalt-dressed pasture rapidly improved in health and eventually became fat. In a report(4) of further work with cobalt compounds for top-dressing cobalt-deficient pastures, mention is made of the satisfactory results obtained from the use of 4 oz. of cobalt chloride at the Marsden Research Farm (which is not a cobalt-deficient area). It was suggested that if the top-dressing with cobalt salts were done in the spring, sufficient cobalt might be made available in the pasture to maintain sheep in health on a cobalt-deficient area, and stated "that the 2 lb. applications used in the experiment" [at Glenhope] "was unnecessarily large and probably could be reduced with safety."

By 1939 it was possible to recommend(5), on the basis of results in the North Island, although these had not been checked by animal trials in the South Island, that 5 oz. of hydrated cobalt sulphate (which is equivalent in cobalt to 4 oz. hydrated cobalt chloride) should be a satisfactory per-acre application if given annually. A large amount of unpublished analytical material has since shown that on the very deficient area at Sherry River 4 oz. of hydrated cobalt sulphate\* per acre produced appreciable increases in cobalt content of the pasture over a useful portion of the season. Following this up with animal trials using wether hoggets, it was shown that over a two-year period two annual applications of 2 oz. cobalt sulphate produced better results than one application at a 4 oz. rate at the beginning of the period(1). It is of importance that even as little as 2 oz. per acre gave such good results on the severely deficient area at Sherry River. In the present paper it has been shown that heavier applications of cobalt sulphate (8 oz. per acre) give better results than 4 oz. per acre over a two-year period. The extra value in weight of meat and of wool on the sheep receiving the 8 oz. application is much greater than the cost of the additional 4 oz. cobalt sulphate applied in the fertilizer.

The position at present, therefore, is that under the conditions of severe cobalt deficiency in the Sherry River district annual dressings of cobaltized fertilizer to provide as little as 2 oz. hydrated cobalt sulphate equivalent per acre will give satisfactory stock health with wether hoggets. Larger applications at 4 oz. and 8 oz. over two seasons have likewise proved satisfactory for hoggets. The larger rate gave the better result. It is to be expected that the best returns would be obtained by increasing the annual applications to at least 4 oz. cobalt sulphate per acre, or to 8 oz. per acre if the dressing is required to be effective over two seasons. The increased yields of carcass and wool would more than pay for the cost of the cobalt in the heavier rates of application.

#### ACKNOWLEDGMENTS

Mr. M. C. Haines and his sons attended carefully to the weighing of the sheep and were responsible for the shearing and other farm attention to the animals. Thanks are due also to Miss J. Watson for analysing the pasture samples and to Mr. A. Schwass for general assistance in the field and laboratory work.

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\* Note that in later work the sulphate has been used instead of the chloride.

## LINEN-FLAX-FIBRE QUALITY IN RELATION TO SOIL TYPES

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*[Received for publication, 21st February, 1946]*

### Summary

An attempt is made to find a method of correlating the quality of linen-flax fibre with the soils on which it is grown. A method by which the degree of development of certain deleterious fibre characteristics is assessed quickly in hand-cut sections is outlined, and an attempt to apply this system of appraisal to the crops from the Geraldine linen flax district in the season of 1943-44 is described.

Correlation of fibre characteristics with soil types shows that each soil type tends to produce fibre of more or less the same quality provided the field influences are the same. Often field influences, such as climate, date of sowing, and nature of the seed-bed appear to be the most important. There was too little variation in the previous agricultural history of paddocks to throw much light on this influence.

On the whole, drought seems to be the principal enemy of good-quality fibre. Any field influence tending to reduce the amount of moisture in the soil (prolonged hot, dry weather, over drainage, droughty soils, or late sowing which brings the latter part of the growing season into the dry January-February period) tends to produce poor fibre. Any influence which makes for uneven distribution of moisture (poor cultivation, leaving large clods of turf with air gaps between, and stony soils) also tends to produce poor and uneven flax.

The fibre index number derived from microscopical examination of the stem sections was also correlated with fibre yields and it was found that a good correlation could be obtained by combining the index number with the stem length. There appeared to be a linear relationship between this composite index and percentage yield of line fibre.

### PURPOSE OF INVESTIGATION

IN an attempt to establish a relationship between soil type on the one hand and fibre quality and yield on the other, a fibre analysis was made of the crops grown in the Geraldine linen-flax district during the 1943 season. This district includes the widest range of soil types in South Canterbury, and at the same time is reasonably accessible to the Washdyke Experimental Factory, where the investigations were carried out.

Microscopic examination of linen-flax sections has hitherto been confined mainly to plant selection, and to the relationship between flax and plant nutrients, and the experiments have been for the most part carried out on the same soil type.

### CLIMATE OF GERALDINE DISTRICT

The crops in the Geraldine district were sown on the downlands and plains within a radius of approximately ten miles from the factory, which is situated about one mile south of the township of Geraldine.

The climate of the Geraldine flax-growing district is typical of the coastal downlands and plains of Canterbury. Rainfalls are low near the coast, and rise very sharply towards the mountains. Average yearly precipitation is

less than 25 in. in the south-east near Temuka, and 48 in. at Orari Gorge. Meteorological information about the district is scanty. Official records are kept at three stations only and are limited to rainfall (Table I).

TABLE I

Station.				Average Annual Rainfall in Inches
Orari Gorge	..	..	..	47.8
Geraldine	..	..	..	29.8
Kakahu	..	..	..	27.8

The prevailing summer wind is the north-easterly, which is forced steeply upwards by the eastern ranges of the Southern Alps. Consequently, a canopy of cloud stretching eastwards from the ranges for a distance of from five to ten miles is a common summer phenomenon. A good deal of light rain is associated with this cloud canopy in the zone immediately adjacent to the ranges: beyond this lies a zone which receives less sunshine than the coastal belt, and in which evaporation is much impeded. The climate of this latter zone seems especially favourable for the growing of linen flax in a normal season. The spring of 1943 was wetter than normal and crops were sown late.

## PART I.—EVALUATION OF FLAX FIBRE FROM GERALDINE DISTRICT

Before the linen flax was sown the fields proposed by the growers for sowing were examined by one of the writers in company with Mr. W. C. L. Leitch, Fields Instructor of the Department of Agriculture, Timaru, and the soil types identified. Records were kept of the condition of the seed-bed throughout the growing season, and of incidence of disease and any other circumstances likely to affect the crops. In January and February, just before the crops, which were all of the Liral Crown variety, were pulled, samples were taken from each and forwarded direct to the experimental factory.

### A. METHOD OF SAMPLING

Two methods of sampling were considered:—

- (1) To use stems collected at random throughout the whole crop during the course of routine appraisal by officers of the Department of Agriculture:
- (2) To collect a fixed length of drill from an arbitrarily selected part of the field. This sample was taken as typical of the greater part of the field.

Dr. Cunningham, Director of the Plant Diseases Division of the Plant Research Bureau, who very kindly undertook to collect samples, recommended and finally adopted the second method.

The field procedure was as follows:—

Three adjacent rows, each 15 ft. in length, were pulled from the inside of the drill width. The outside rows of each drill width were therefore discarded. The sheaves were retted, scutched on the Van Hauwaert scutcher, and the fibre percentage obtained. Three samples, composed of stems collected at random over the whole field, were also taken: one from crop 122\*

\* Crop numbers are given in Table XV.



taken by officers of the Department of Agriculture and one each from crops 116 and 129 taken by the writer.

Before retting, fifty stems were chosen from each sheaf, by taking nine or ten groups of any five or six stems.

A piece of stem about 2 in. long was cut from immediately below the point midway between the first branch of the inflorescence and the stem at ground-level. The lower end was cut obliquely for identification. Sections from the midway point are those most usually chosen by overseas workers and are apparently the most satisfactory.

## B. PREPARATION OF SECTIONS

The stems were fixed in form-acetic-alcohol: 70 per cent. ethyl alcohol, 90 ml.; glacial acetic acid, 5 ml.; formalin, 5 ml.

The stems were cut unimbedded, using the small brass section-cutter developed at Lambeg and described in L.I.R.A. publications Nos. 2 and 5. It consists of a body-piece, with a detachable screw head. Through the body-piece and screw head is a cylindrical hole either  $\frac{3}{16}$  in. or  $\frac{1}{4}$  in. in diameter. A screw at the bottom works a plunger up through the hole. A piece of elder pith is compressed by rolling until it is small enough to fit into the screw head. A hole of appropriate diameter is made through the pith with a needle or drill and the flax stem inserted. The pith is placed in the screw head and moistened to make it fit tightly around the stem and into the head. The pith and stem are then extended to the desired distance and the extruded part cut off by a razor. The sections were mounted in 50 per cent. glycerine and water.

## C. PROCEDURE FOR MAKING FIBRE COUNTS

Two methods of evaluating fibre characteristics were tried, the first by exact measurement of fibre diameters and numbers on a projected image of the section, and the second by a direct microscopic assessment of a number of fibre characteristics.

### (a) BY PROJECTION AND EXACT MEASUREMENT

Exact measurements of fibre dimensions and a series of counts of average numbers of fibres per bundle were made. From previous work it was deemed desirable to measure at least thirty stems, or more, if possible, from each crop: fifty was finally chosen as being the greatest number that could be conveniently dealt with.

The sections were projected by means of a lamp housed in a wooden box 24 in. square and 22 in. high with an open front, and examined through a microscope placed horizontally. The image was reflected upwards by a plate-glass mirror at an angle of  $45^\circ$  on to a horizontal piece of plate glass to which was pasted a piece of tracing-paper.

The magnifications, expressed as linear diameters, used for measurement of percentage of fibre in the stem, were 177X for the larger and 230X for the smaller stems; the magnification for average fibre size was 1340X and for size of lumen 2140X.

The percentage of fibre computed as percentage of the area of the cross-section does not necessarily give the percentage of fibre as weight in the stem, since no account is taken of the specific gravity of the substances in the tissues nor the amount of space taken up by the cell lumina. However, it was considered that a suitable measurement of areas should suffice for a comparison between crops, rather than determination of the absolute percentage of fibre by weight.

Five measurements were made as follows :—

(1) AREA OF FIBRE AS A PERCENTAGE OF STEM AREA

(Referred to henceforth for brevity as " fibre in stem area ")

The chief objection to this measurement is that it ignores the possibility that the proportion of the stem occupied by the pith may vary considerably. Since the pith in the mature stem has usually almost completely disintegrated and is at any rate an extremely light tissue compared with the xylem, this measurement may be misleading. On the other hand, it is the easiest to make.

(2) PERCENTAGE OF FIBRE IN THE AREA OF THE STEM MINUS THE PITH

(Referred to henceforth as " fibre in the stem minus pith ")

This measurement should give a more suitable figure for comparison between crops as it allows for the size of the pith.

(3) THE SIZE OF THE LUMEN EXPRESSED AS A PERCENTAGE OF THE AREA OF THE FIBRE

It was known that the wall thickness of flax fibres varies and that the percentage of fibre measured by area might be misleading unless allowances were made for this variation. Moreover, the wall thickness might be expected to have a bearing on the strength and optical properties of the fibres.

In each stem three fibres—one from the outside, one from the middle, and one from the inside of the bundles used for fibre size—were measured for lumen size.

(4) AVERAGE FIBRE SIZE

A typical bundle from each stem was selected, the fibres counted, and the average size of the fibres calculated.

(5) THE AVERAGE NUMBER OF FIBRES PER BUNDLE

The total number of fibres was counted and divided by the number of fibre bundles.

Since the method is slow (seven to eight days per crop), it was thought best to try it out first on some representative crops. Accordingly four crops (137B and 98A, representing the better crops, and 123 and 108 the poorer) were selected and examined. Average fibre size measurements were made on ten stems, and the remaining measurements on fifty stems.

Although the crops chosen obviously showed a wide range of quality the differences in measurements were not correspondingly significant. It was felt, therefore, that the value of the method did not justify the time it consumed. Moreover, it did not take into account such clearly important fibre characters as fibre shape, &c. The method was therefore discarded in favour of another which gave less accurate measurements of fibre dimensions but a more comprehensive picture.

## (b) DIRECT MICROSCOPIC METHOD

It was thought that by concentrating on more easily recognized fibre and stem characteristics by direct microscopic examination a quicker rough estimate might be made of the amount and quality of fibre in the stem.

Accordingly, some eight easily recognizable fibre characteristics were listed and their degree of development estimated. For this a system of notation was evolved in which the degree of development of the particular characteristic was indicated by a number, as indicated below.

The classification assigned to a crop under each index was based on ten sections selected at random from the fifty and classified individually. In cases of doubt, another group of about ten was considered in conjunction with the first ten.

## (1) FIBRE CHARACTERS CHOSEN

I. *Bundle Shape*

(1) *Regular*.—The bundles are regular and fairly smooth in outline; the shape is oblong to oval.

(2) *Slightly Irregular*.—The bundles are less smooth in outline and deviate a little from the regular shape of Class 1.

(3) *Very Irregular*.—The bundles are irregular and show marked projections and constrictions.

There would seem to be a greater chance of loss of fibre from irregular bundles in retting and scutching, since the bundles would be more readily broken up or the projecting fibre broken off. Regular bundles would give a more uniform fibre.

II. *Evenness within the Bundle*

(1) *Even*.—The fibres within the bundle are fairly even in size and usually tend to lie evenly in rows.

(2) *Mixed*.—The fibres within a bundle vary considerably in size, and fibres of different sizes are scattered throughout the bundle.

(3) *Two Zones*.—In some stems the fibres show a division into two zones. The inner fibres differ in shape from the outer ones, and, so far as has been noted, are always poorer in shape, being "squashed" or "folded" (see Index III); often, but not always, the layering is more marked and the fibres thinner and larger.

The outer fibres of each bundle are the first to develop wall thickening; the inner ones develop later. An inner zone of poor fibres shows, therefore, that the later part of the growing season was unfavourable for the growth of good fibre.

The even bundles, which are the most uniform, are given the highest grade, and the two-zoned, which are least uniform, the lowest.

III. *Fibre Shape*

(1) *Polygonal*.—The fibres are polygonal in outline, either regular hexagons or irregular polygons. The intercellular spaces are absent or only very small.

(2) *Rounded*.—The fibres are round in outline. There are intercellular spaces.

(3) *Squashed*.—The fibres are somewhat flattened and elongated tangentially; the corners are rounded.

(4) *Folded*.—The cell wall is in places folded inwards forming a re-entrant angle or the whole fibre is flattened and bent.

Fibre shape is the fibre characteristic which has been used hitherto principally by German workers to measure fertilizer response in linen flax. Closely packed fibres, polygonal in cross-section, are regarded as ideal. The various degrees of departure from this are set out above.

#### IV. Layering

(1) *Faint*.—The layering is very faint.

(2) *Moderate*.—The layers are moderately well marked, or there is not more than one strongly marked one.

(3) *Strong*.—The layering is very strongly marked.

(4) *Clearage*.—There is separation between layers of the wall. In cases where the cell wall is thick and the only cleavage is shown by an exceedingly thin layer separating next to the lumen, this is disregarded.

The fibre walls are made up of a number of layers, the development of which is considered undesirable, since, when these are most strongly marked, there may be actual separation between the layers, which, it may be supposed, would lead to lack of strength in the fibre.

#### V. Wall Thickness

(1) *Thick Walls*.—The walls are thick and the lumen almost filled.

(2) *Moderate Walls*.—The walls are thinner and the lumen somewhat larger.

(3) *Thin Walls*.—The walls are thinner and the lumen takes up about one-third or more of the diameter of the fibre or one-third the width of the short axis in a flattened fibre.

The thinner-walled fibres are given a poorer classification since they must be lighter and weaker than the thicker fibres.

The cells may be so thick that the lumen is almost completely filled or so thin that it is little thicker than an ordinary parenchymatous cell. No crop showed stems with a large number of extremely thin walled cells, and no classification is included for them here; they are taken into account in the "percentage of poor fibres."

#### VI. Fibre Size

(1) *Large*.—Average measurement of the diameters of five fibres is 0.095 mm. or over. There were nine crops in this class.

(2) *Medium*.—The average measurement of the sums of the diameters of five fibres is 0.080 mm. and up to 0.095 mm. There were twenty-three crops in this class.

(3) *Small*.—The average measurement of the sums of the diameters of five fibres is less than 0.080 mm. There were seven crops in this class.

To measure fibre size the length of a tangential row of fibres was measured, using a camera lucida. In each stem three measurements were made, all in the same bundle where possible, of a row on the outside, middle, and inside of a bundle. The smallest group of fibres measured was 0.053 mm. and the largest 0.134 mm. long. The lowest average was 0.072 mm. and the highest 0.104 mm.

The difference between the larger and the smaller fibres does not seem to be great enough to affect the spinning quality of the flax, and no effect seems to be known. Since larger fibres give a larger quantity of flax they are counted as more desirable than small.

### VII. *Number of Fibres per Unit Area of Stem*

- (1) Many fibres per unit area of stem.
- (2) A moderate number of fibres per unit area of stem.
- (3) Few fibres per unit area of stem.

Within the same crop the number of fibres per stem on the whole increases with the diameter of the stem; but if stems of the same diameter from different crops are compared the number of fibres per stem may be very different, as seen in the three crops shown in the table below.

TABLE II.—TOTAL NUMBER OF FIBRES

Stem Diameter			Crop 96.	Crop 125	Crop 95
0.95 mm. ..	..	..	283	398	506
1.55 mm. ..	..	..	474	579	766

This index, referred to for convenience as “amount of fibre,” should, combined with that of fibre size, give a rough indication of the amount of fibre in the stem.

### VIII. *Percentage of Poor Fibres*

Fibres with extremely thin walls, “folded” fibres with thin walls, and fibres with a very high degree of cleavage are classed as poor.

The percentage of poor fibres in any stem ranged from 0.38 per cent. The smallest percentage in any crop was 0.1 per cent. and the highest 19 per cent.

The classes are—

- (1) 0.1 per cent.
- (2) 1–3 per cent.
- (3) 3–10 per cent.
- (4) Over 10 per cent.

The poor fibres include all those that are weak and light and many which are possibly easily lost in processing and therefore undesirable.

Fibres of this type may occur in all stems. When a few of these poorer fibres are scattered among better fibres they are insufficient to class the stem in an extra class “extremely thin walls” or in “folded,” but they cannot be disregarded. They are therefore treated in a separate class.

## (2) FIBRE INDEX NUMBER

For purposes of general comparison the sum of the individual indices has been used.\* This sum is henceforth referred to as the "fibre index number." It gives a handy way of arranging the crops according to the picture presented by the fibre under the microscope.

The crops sectioned are shown with their fibre index numbers and their classification under each index in Table IV.

## (3) SOME FEATURES CONSIDERED AS INDICES BUT DISCARDED

(a) *Average Number of Fibres in the Fibre Bundle*

This was measured for ten stems in each crop and the average taken over the ten stems.

(b) *Total Number of Fibres in the Stem*

The total number of fibres in ten stems was counted and averaged. These two indices—(a) and (b)—were chosen to give some idea of the amount of fibre in a crop. For the most part they measure the same thing in different ways, as will be seen from Table III.

TABLE III.—AVERAGE NUMBER OF FIBRES IN THE BUNDLE

Number of Fibres in Stem	14-15	16-17	18-19	20-21	22-23	24-25	26-27
801-850 .. ..	..	..	..	..	..	1	..
751-800 .. ..	..	..	..	1	..	..	..
701-750 .. ..	..	..	..	..	..	..	1
651-700 .. ..	..	..	..	1	..	2	..
601-650 .. ..	..	..	..	3	..	..	..
551-600 .. ..	..	..	3	4	2	..	..
501-550 .. ..	..	2	9	1	..	..	..
451-500 .. ..	..	7	1	..	..	..	..
401-450 .. ..	1	2	..	..	..	..	..
351-400 .. ..	2	..	..	..	..	..	..

However, some crops that would come in the highest class in (b) come in a lower class in (a), for when the number of fibres is over 750 there is often a greater number of bundles than would be expected. "Total number of fibres" is therefore a better measurement than "average number of fibres in bundle."

Neither of these indices takes into account the stem diameter or the fact that the number of fibres in a stem tends to increase with an increase in stem diameter. Thus two crops differing greatly in stem diameter may on a fibre count be put in the same class, whereas the actual number of fibres per unit area of the stem may be very different - e.g., crop 106. The stems were fairly fine and on fibre count they fell into Class 4, with 501-600 fibres, whereas taking their diameter into account they come into Class 1 in the index, "amount of fibre."

\* In a few crops which have two zones two values are recorded for some characteristics, one for the inner and one for the outer zone. When these are widely different, as for layering in crop 102 (with outer zone 1 and inner zone 4) an intermediate value is used in assessing fibre index number.

*(c) Number of Rows in the Bundle*

The fibres are arranged in the bundles roughly in rows. It is obvious that, other things being equal, the greater the number of rows in the bundles, the greater the percentage of fibre.

This was merely a rough way of estimating the same thing as the two preceding indices, since those stems with one to two and two to three rows in the bundle for the most part fell into classes with a low number of "total fibres" and "average bundle size," those with three to four rows in classes with a large number of total fibres and large average bundle size.

*(d) Average Number of Fibres per Bundle*

The crops were put by estimation into three classes—with many, a moderate number, and few fibres per bundle. The results obtained by estimation agreed reasonably well with those obtained by actual counting for total number of fibres in the stem.

The four preceding indices were discarded in favour of VII, "Number of fibres per unit area of stem."

*(e) Difference in Two Sides of the Stem*

There is sometimes a difference in size, occasionally marked, between the fibre bundles on two sides of the stem. The difference may be due to difference either in size of the individual fibres or the number of radial rows of fibres in the bundle. Three classes were made, where the difference was lacking, slight, or great. The index was discarded because observations made on different sets of stems from the same crop did not agree.

*(f) Fibre Shape*

Originally the class "polygonal" was divided into two—"regular hexagons" and "polygonal"; but "regularly hexagonal" occurred with "even fibres" in Index IV and "polygonal" with "mixed," as was to be expected. Since the same feature was being taken into account twice over, the distinction between fibre shapes was dropped.

**(4) SOME FEATURES NOTED BUT NOT INCLUDED IN THE INDEX***(a) Ratio of Stem to Pith*

The proportion occupied by the pith varies in different stems. Since the pith almost completely disintegrates and is, at any rate, an extremely light tissue, the greater the proportion of stem occupied by pith, the greater the percentage of fibre obtained by weight from the stem, other things being equal.

In ten stems from each crop the ratio of the diameter of the stem to the diameter of the pith was measured under the camera lucida and the results averaged. Table IV contains the results for the lowest and highest averages of ratios found in individual stems; it shows the ratio of the area occupied by tissues other than pith to the area of the pith calculated from the measured diameters. The calculations indicate that the other tissues may occupy less area than the pith, as in a stem affected by false browning, or up to or over four times the area of the pith in a stem from crop 122.

The method of measuring is rough, and the ratio calculated from these measurements differs by 1-10 per cent. from that obtained on the same stem by planimeter reading on the projected magnified image. However, extremely woody stems can be satisfactorily distinguished from hollow stems.

TABLE IV

Crop	Stem/Pith Ratio	Ratio Area of Tissues to Area of Pith.
<i>Average Ten Stems</i>		
102 (much false browning) ..	1.49	1.22
129 .. ..	1.61	1.59
134B .. ..	2.16	3.66
<i>Single Stem</i>		
102 (much false browning) ..	1.39	0.74
129 .. ..	1.52	1.31
122 .. ..	2.40	4.76

### (b) Rings in Wood

Usually the vessels of the xylem become progressively smaller from the pith to the cortex, but in the xylem of some stems there is an interesting ring of larger vessels which seems to indicate an unevenness in the growth of the plant. This unevenness, so far as is known, is not reflected in the fibre.

### (5) COMMENTS ON EACH FIBRE CHARACTERISTIC

#### *Layering and Percentage of Poor Fibres (Table V)*

As layering does not come into the definition of a poor fibre it can only be correlated with poor fibres as far as it affects wall thickness or fibre shape, or both. None with faint layering had more than 1 per cent. of poor fibres. One sample, that showing false browning, had moderate layering and more than 10 per cent. of bad fibres: opposite extremes did not occur together.

TABLE V.—LAYERING AND PERCENTAGE OF POOR FIBRES

	Class	Layering.			
		1.	2.	3	4
Percentage of poor fibres {	4	..	1	..	2
	3	..	3	2	1
	2	..	3	1	1
	1	5	10	13	..



*Layering and Fibre Shape (Table VI)*

Cells with polygonal fibre shape show layering "faint" to "strong"; "squashed" cells show layering "strong" to "cleavage"; "folded" cells show "moderate" layering and "cleavage." Good cell shape shows everything except the worst layering: poor cell shape generally, but not always, is associated with strongly developed layering.

TABLE VI.—LAYERING AND FIBRE SHAPE

	Class	Layering.			
		1.	2.	3	4
Fibre shape	4	..	1	..	2
	3	..	..	2	2
	2	..	3	8	..
	1	5	12	5	..

*Layering and Wall Thickness (Table VII)*

The thick-walled fibres have faint to moderate layering, and thin-walled fibres have moderate layering to cleavage. The layering tended to increase with thinness of the wall. In no case is there extremely good quality in one with extremely bad quality in the other, but the two qualities are not so strongly associated that one can be used as a measure for the other.

TABLE VII.—LAYERING AND WALL THICKNESS

	Class	Layering			
		1	2	3	4.
Wall thickness	3	..	1	3	4
	2	2	11	12	..
	1	4	7	1	..

*Layering and Evenness within the Bundle (Table VIII)*

Both even and uneven bundles show faint to strong layering. Two-zoned bundles show moderate layering to cleavage, the cleavage when present being in the inner zone. Opposite extremes did not occur together. There seems no strong tendency for degree of layering to be associated with unevenness within the bundle.

TABLE VIII.—LAYERING AND EVENNESS WITHIN THE BUNDLE

	Class.	Layering.			
		1.	2.	3.	4.
Evenness within bundle	3	..	2	3	4
	2	2	14	12	..
	1	3	1	1	..

*Fibre Shape, Wall Thickness (Table IX)*

None with thick walls have "squashed" or "folded" fibres. Thin-walled cells could have any shape.

TABLE IX.—FIBRE SHAPE, WALL THICKNESS

	Fibre Shape.				
	Class.	1	2	3	4
Wall thickness	3	2	1	1	2
	2	13	10	4	.
	1	9	2	..	..

*Percentage of Poor Fibres and Wall Thickness (Table X)*

Since a fibre with extremely thin walls is classed as poor it is not surprising to find that all crops in Class 4, "Percentage of Poor Fibres," come into Class 3, "Thin Walls" for fibre thickness, and that no crops classed "thick walled" have more than 3 per cent. of poor fibres. A high percentage of poor fibres occurs only in thin-walled stems, but a low percentage of poor fibres may be found with any class of wall thickness.

TABLE X.—PERCENTAGE OF POOR FIBRES AND WALL THICKNESS

	Percentage of Poor Fibres				
	Class	1	2	3	4
Wall thickness	3	3	..	1	3
	2	17	3	5	..
	1	7	3	..	.

*Percentage of Poor Fibres and Evenness within the Bundle (Table XI)*

All with a high percentage of poor fibres have two zones and all with a high degree of evenness within the bundle have a low percentage of poor fibres; but "two zones" does not necessarily go with a high percentage of poor fibres nor a low percentage of poor fibres with extreme evenness.

TABLE XI.—PERCENTAGE OF POOR FIBRES AND EVENNESS WITHIN THE BUNDLE

	Percentage of Poor Fibres				
	Class	1	2	3	4
Evenness within bundle	3	1	2	3	3
	2	22	3	2	..
	1	5	..	..	..

*Percentage of Poor Fibres and Bundle Shape (Table XII)*

There is no association between bundle shape and the percentage of poor fibres.

TABLE XII.—PERCENTAGE OF POOR FIBRES AND BUNDLE SHAPE

		Percentage of Poor Fibres.				
		Class.	1.	2.	3.	4.
Bundle shape	{	3	5	2	4	1
		2	20	4	1	2
		1	1	..	..	..

*Fibre Size and Layering and Fibre Size and Wall Thickness (Tables XIII and XIV)*

No association of fibre size with either layering or wall thickness is shown.

There was no association between bundle shape and any other feature.

The results show that although there is sometimes correlation between the qualities of different indices it is not enough to justify discarding any index.

TABLE XIII.—LAYERING AND FIBRE SIZE

		Layering				
		Class.	1.	2.	3.	4.
Fibre size	{	42-43	..	1	2	1
		40-41	..	1	3	..
		38-39	..	4	4	..
		36-37	1	2	4	2
		34-35	..	2	2	1
		32-33	3	4	1	..
		30-31	1	3	..	1

TABLE XIV.—WALL THICKNESS AND FIBRE SIZE

		Wall Thickness.			
		Class.	1.	2.	3.
Fibre size	{	42-43	1	1	3
		40-41	..	3	1
		38-39	2	4	1
		36-37	2	5	2
		34-35	2	3	..
		32-33	3	6	..
		30-31	1	2	2

D. RELATIONSHIP OF THE FIBRE INDEX NUMBER AND STEM  
LENGTH WITH FIBRE PERCENTAGE

Since ultimate fibre quality, after processing is completed is determined as much by the hazards of processing and the history of the crop between its maturity and the time the factory receives it, as by the quality of the fibre at maturity, exact correlation between the grades assigned to the processed fibre by the grader and the fibre index number was not expected, nor was it found. The final grading showed practically no correlation with

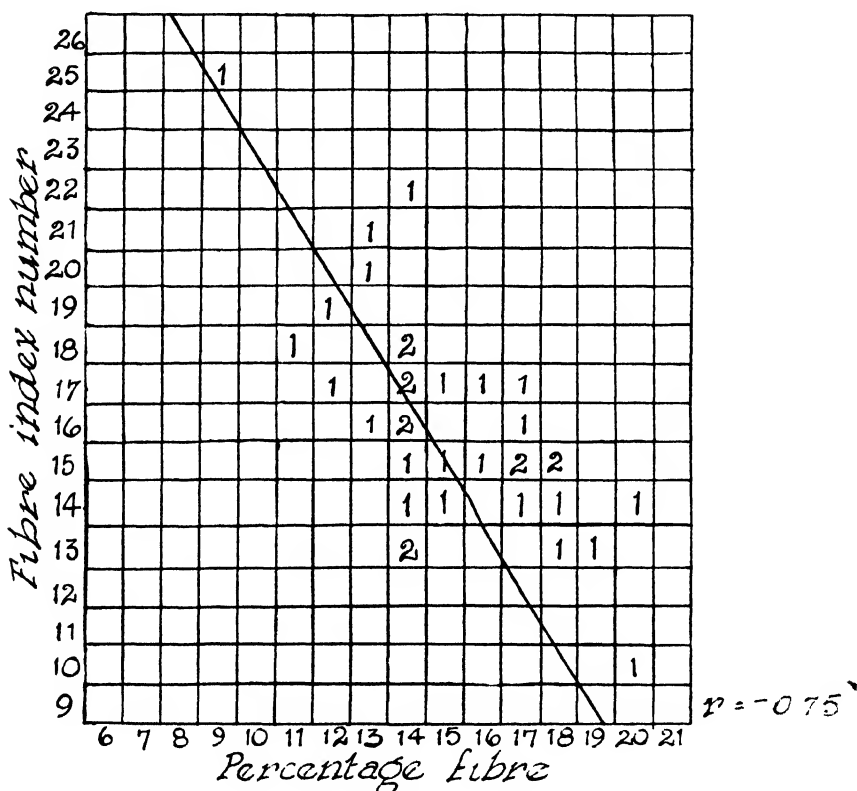


FIG. 1.

any field factor. Any attempt to link the index with the spinner's evaluation of the flax was felt to be unsatisfactory for the same reasons. Instead, the index was shown to be correlated with quantitative yields of line fibre.

The fibre percentage and fibre index graph (Fig. 1) shows that fibre percentage increases with decreasing fibre index number. There is moderately good correlation, the coefficient of correlation being  $-0.75$ .

The fibre percentage and stem length graph (Fig. 2) shows that as the stem length increases the fibre percentage increases. The correlation coefficient is  $0.75$ .

Stem length appears to have an effect on percentage yield of line fibre comparable with that of the derived fibre index number. Therefore it was thought that by combining stem length with the fibre index number into a new composite index a higher correlation might be got with fibre percentage. This new index was derived by adding to the fibre index number the difference between fibre length and 30 in., which was taken as a more or less arbitrary optimum length. This new index was plotted against fibre percentage and the graph (Fig. 3) showed a fairly well-defined linear relationship and a higher correlation coefficient ( $-0.82$ ).

Since the fibre index number is based on certain selected features only, perhaps not all of equal significance, and since fibre yields are likely to be altered considerably during the largely uncontrolled process of bacterial

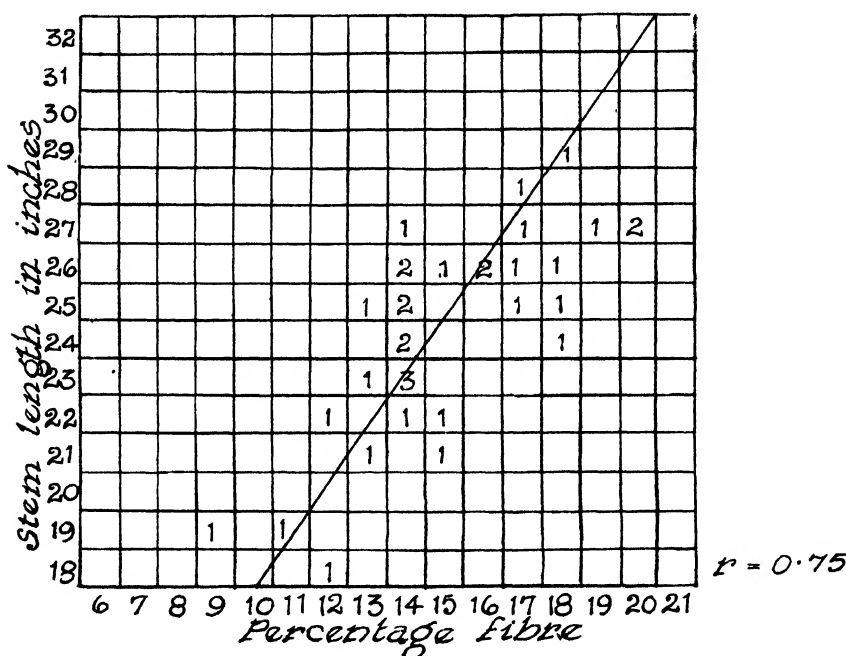


FIG. 2.

retting and subsequent processing, it is not to be necessarily supposed that there should be a perfect correlation between fibre index number and fibre yield. Errors may be introduced in sampling. During scutching some fibre may have been unnecessarily lost as tow, but no records were kept of tow yields and therefore fibre percentage yields are incomplete.

Some of the scattering may be due to the differences in the stem/pith ratio, which is not included in the fibre index. The stem/pith ratio of most of the samples varied very little, but two samples (117 and 134B) with abnormal ratios have been omitted from the above analysis. 117 and 134B had each abnormally high stem/pith ratios, which lowered the expected percentage yield.

## PART II.—FLAX-GROWING SOILS OF GERALDINE DISTRICT

Only those soil types on which linen flax was sown are discussed. Seven soil types are concerned, and a brief description of each follows.

### SOIL TYPE 3. ORARI SILT LOAM

Orari silt loam is derived from greywacke alluvium. It is only moderately leached, and is well supplied with plant nutrients. In texture the top soil ranges from a silt loam to a sandy-silt loam. Textures sandier than this are relatively rare. Subsoils are slightly heavier than topsoils and moderately well drained except in depressions. Gravels lie at from 3 ft. to 5 ft. below

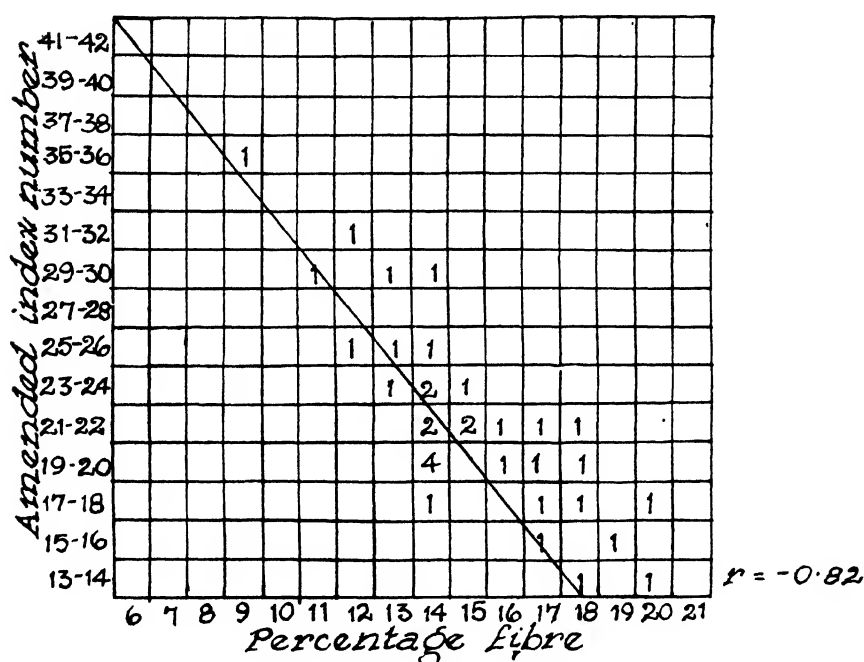


FIG. 3

the surface. Orari silt loam is sufficiently well drained to allow early sowing of flax, and at the same time it has good moisture-holding properties. It is therefore very suitable for the growing of linen flax.

### SOIL TYPE 3C, ORARI SILT LOAM, MODERATELY SHALLOW PHASE

The moderately shallow phase of Orari silt loam differs from Orari silt loam mainly in its greater degree of stoniness, lower fertility, shallower profile, and freer drainage. It tends to be droughty. Gravels lie at from 16 in. to 24 in. below the surface. To grow successful flax it needs plenty of evenly distributed rainfall throughout the growing season, or irrigation.

#### SOIL TYPE 3D, ORARI SILT LOAM, SHALLOW SANDY PHASE

The shallow sandy phase of Orari silt loam differs from Orari silt loam in its shallower profile and sandier texture. The texture ranges from a coarse, very sandy loam resting on sands to coarse sandy loam on gravels. The gravels are usually within 18 in. of the surface. Gravel ridges are common, and on these flax is rarely successful. False browning is mostly restricted to such shallow ridges. The shallow sandy phase of Orari silt loam is overdrained and droughty, and for successful flax-growing it would require excessive rain well distributed throughout the growing season, or irrigation. In a normal South Canterbury season it would be classed as unsuitable for flax.

#### SOIL TYPE 4A, WAITOHI SILT LOAM, WELL-DRAINED PHASE

Waitohi silt loam is derived principally from loessial parent material, and like all other soils derived from the same parent material it has a silt-loam texture. It is typical of the higher terraces of the plains and may be regarded as one of the oldest alluvial soils in South Canterbury. It is strongly leached and fertility is on the low side. There is a moderate lime deficiency. The texture of the topsoil is invariably a silt loam. Subsoils are heavier than topsoils, ranging from heavy silt loams to silty clay loams. Through-drainage of the profile is poor, but the surface is gently undulating and surface drainage is good.

#### SOIL TYPE 4B, WAITOHI SILT LOAM, POORLY-DRAINED PHASE

The poorly-drained phase of Waitohi silt loam differs from Waitohi silt loam (soil type 4A), mainly in its flatter surface and therefore poorer surface drainage. In a wet spring, therefore, cultivation is delayed, and heavy rain at harvest may seriously impede pulling. Overcropping readily destroys its structure and makes it even more easily waterlogged. It has less plant foods than type 4A, lime being especially deficient. Two crops were rejected on account of short stems.

#### SOIL TYPE 8A, TAIKO SILT LOAM

Taiko silt loam is typical of the rolling downlands where the soils are derived from loess. It is moderately leached, with a subsoil heavier than the topsoil. Surface drainage is good, but drainage through the profile is poor. In wet weather, therefore, cultivation may be impeded. Prolonged rain may seriously delay sowing and pulling.

It is moderately well supplied with plant foods and has only a moderate lime requirement. The structure is a soft crumb which readily deteriorates under intensive cultivation.

#### SOIL TYPE 8C, TAIKO SILT LOAM, LEACHED PHASE

The leached phase of the Taiko silt loam lies under a slightly higher rainfall and a much greater humidity. It has developed on the downlands near the ranges, and is much more strongly leached than Taiko silt loam. It has less plant foods than type 8A, but none of these appear to be deficient enough to limit the growing of flax.

**PART III.—RELATIONSHIP BETWEEN SOIL TYPES AND FIBRE QUALITY**

The crops which were grown on each soil type, together with details of their fibre indices, are shown in Table XV.

**(1) FIBRE CHARACTERISTICS OF FLAX GROWN ON SOIL TYPE 3 (ORARI SILT LOAM)**

*Quality index = 12.* Only two crops were sown on Orari silt loam, and both showed marked similarity in fibre picture. The two crops differed principally in the number of fibres in the stem (groups 1-2). In all other respects the fibre picture was practically the same. According to the system of classification used both crops were of high quality.

Orari silt loam may be regarded as the best of the linen-flax-growing soils of the Geraldine district. Approximately 4.0 per cent. of the total acreage of flax in the district was sown on this type.

**(2) FIBRE CHARACTERISTICS OF FIBRE GROWN ON SOIL TYPE 3c (ORARI SILT LOAM)**

*Quality index = 16.4.* Five crops were sown on Orari silt loam, and these show a moderate degree of variation. They differ principally in the fibre size, which ranges from group 1 to group 3.

The number of fibres per bundle, bundle shape, layering, and evenness in bundle are all medium, and range from group 2 to group 3.

All crops show a small percentage of poor fibres, except 131B, which has a high percentage, falling into group 3.

The best fibre index number is that of crop 115 [14].\* This was to be expected, since the soil type on which it was sown approaches type 3, which grew crops with an index of 12.

The worst crop was 130, with an index of 19. This was sown on a rather shallower phase, some of the paddock being excluded from sowing on account of its extreme stoniness. This field was easily the most droughty of the five.

Crop 134B, with a fibre index number of 17, gave an exceptionally low yield of line fibre. This may be explained by the high ratio of wood to pith, which upset an otherwise fair fibre picture. This crop moreover had a higher proportion of bad fibre.

The effect of date of sowing shows fairly well on this soil type :—

Date of Sowing Index.*					Crop No.	Fibre Index No.
4	..	..	..	..	115	15
4	..	..	..	..	130	20
5	..	..	..	..	98A	16
6	..	..	..	..	134B	17
6	..	..	..	..	117	18

\* Date of sowing: 4, 15th to 31st October; 5, 1st to 14th November, 6, 15th to 30th November.

**NOTE.**—The fibre index number of crop 130 is high because of extreme droughtiness. Crop 134B was rejected because its average length was less than 20 in. The cultivation of the seed-bed for 117 was poorer than for 134B.

\* Numbers in square brackets refer to crop index numbers.



TABLE XV

Soil Type.	Crop No.	Bundle Shape.	Evenness within Bundle.	Fibre Shape.	Layering.	Wall Thickness.	Fibre Size.	Number of Fibres per Unit Area Stem.	Percentage Poor Fibres.	Stem: Pith Ratio.	Fibre Index No.	Stem Length in Inches.	Percentage Line Fibre in Sheaf.	Date of Sowing Index*.	Crop No.
3	129	2	1	1	1	1	2	1	1	1.61	10	22	..	3	129
	116	2	1	1	1	1	2	2	1	1.62	11	..	..	3	116
3C							Average	..		1.61	10.5				
	115	3	2	1	1	1	3	2	1	1.89	14	22	7.7†	4	115
	98A	2	2	1	3	2	2	2	1	1.78	15	25	13.9	5	98A
	117	2	2	2	3	3	1	3	1	2.00	17	24	10.2	6	117
	134B	2	2	2	2	2	2	2	3	2.16	17	21	6.5	6	134B
	130	3	3	3	3	2	2	2	1	1.80	19	18	11.7	4	130
3D							Average	..		1.92	16.4	22	10.5 12.0‡		
	102	2	3	2, 4	1, 4	3	2	1	4	1.62	21	20	14.4	4	102
4A	120B	2	1	1	3	2	1	2	1	1.84	13	27	18.7	4	120B
	120A	2	2	1	2	2	2	2	1	1.79	14	27	19.8	5	120A
	127	2	2	1	2	2	2	2	1	1.91	14	24	14.0	6	127
	125	2	2	2	3	2	2	2	1	1.77	15	27	17.1	5	125
	125	2	2	2	2	2	2	2	1	1.93	15	26	15.6	5	125
	126	2	2	2	3	2	1	3	1	1.82	16	25	16.8	5	126
	121	2	2	2	3	2	2	3	1	1.96	17	22	11.8	6	121
							Average	..		1.86	14.8	25.4	16.2		

\* Date of sowing: 3, before 15th October, 4, 15th to 31st October, 5, 1st to 14th November, 6, 15th to 31st November. Some of fibre lost in scutcher. Omitted from average. ‡ Average with 117 and 134B omitted. † Minimum.

TABLE XV—continued

Soil Type.	Crop No.	Bundle Shape.	Evenness within Bundle	Fibre Shape	Layering	Wall Thickness	Fibre Size	Number of Fibres per Unit Area Stem	Percentage Poor Fibres	Stem Pith Ratio	Fibre Index No.	Stem Length Inches	Percentage Fibre in Sheaf	Date of Sowing Index.*	Crop No.
4B	100	2	2	1	3	2	2	3	1	1.91	15	21	15.2	5	100
	132	2	2	2	3	2	1	2	1	1.79	15	26	16.8	5	132
	99	2	2	1	3	3	1	3	1	1.84	16	..	..	4	99
	104	3	3	2	3	2	2	2	1	1.78	16	23	14.2	5	104
	118	3	3	2	3	2	2	3	2	1.86	17	23	13.7	5	118
	133A	3	3	1	3	3	1	3	1	1.71	17	27	14.3	5	133A
	112	3	3	1	3	2	2	3	1	1.74	17	26	15.5	5	112
	123	3	3	1	2	2	3	2	3	1.84	18	19	10.7	5	123
	108	3	3	1, 3	3	2	2	2	3	1.74	20	21	13.4	5	108
	96	3	3	3	4	2	1	3	3	1.66	22	23	14.1	5	96
	135	3	3	2, 4	4	3	2	3	4	1.79	25	19	9.1	6	135
							Average			1.79	18.0	22.8	13.7		
8A	107	2	2	1	2	1	2	2	1	1.81	14	24	14.2	5	107
	106	2	2	1	2	1	3	1	2	1.70	14	22	15.2	5	106
	131A	3	3	1	2	1	2	2	1	1.72	14	26	17.5	4	131A
	97	2	2	4	3	2	2	2	1	1.82	15	25	17.6	5	97
	101	2	2	2	3	2	2	2	2	1.72	17	26	14.9	5	101
	95	3	3	1	3	2	3	1	2	1.74	18	22	14.2	5	95
	124	2	2	1, 3	4	2	2	1	2	1.68	18	26	13.6	5	124
							Average			1.75	15.7	24.4	15.3		
8C	114	1	1	1	1	1	3	1	1	1.76	10	27	19.7	5	114
	137B	2	2	1	2	1	1	3	1	1.88	13	29	14.4	5	137B
	137A	2	2	1	2	1	2	2	1	1.76	13	26	18.3	5	137A
	122	2	2	1	3	1	1	3	1	2.04	14	..	..	6	122
	109	2	2	1	2	2	3	2	1	1.88	15	24	17.6	6	109
							Average			1.86	13.0	26.5	17.5		

\* Date of sowing: 3, before 15th October 4, 15th to 31st October 5 1st to 14th November 6, 15th to 30th November

Approximately 17 per cent. of the total area was sown on the moderately shallow phase of Orari silt loam. It may be regarded as a problem soil, and its linen-flax potentialities should be further investigated. A small potash trial on a portion of crop 130, where potash was top-dressed at the rate of  $1\frac{1}{4}$  cwt. to the acre, showed a distinct improvement in fibre quality.

(3) FIBRE CHARACTERISTICS OF FIBRE GROWN ON SOIL TYPE 3D (ORARI SILT LOAM, SHALLOW SANDY PHASE)

*Quality index = 21.* Only one crop (No. 102) was sown on this soil type. This crop had a good deal of false browning on shallow stony ridges. Samples were taken from the patches of false browning and also from the remainder of the paddock, which showed no visible deterioration. Both samples gave an almost identical picture, and suggested that the browning was only the final stage of a more fundamental change that had taken place earlier, and that before the stem actually died off, the droughty conditions had already had a detrimental effect on the fibre.

All undesirable fibre characteristics were well developed in both samples, with the percentage of poor fibres exceptionally high. (In the false browning samples the inner fibres were poorly developed, probably owing to the plant having died off earlier. The cleavage lines were not so prominent, probably for the same reason.)

This crop might have been better if it had been sown earlier, when it would have escaped the droughty part of the season from late December onwards. The shallow sandy phase of Orari silt loam appears to be unsuitable for growing linen flax, and if it is used the flax should be sown as early as possible.

Approximately 1.0 per cent. of the total area was sown on the shallow sandy phase of Orari silt loam.

(4) FIBRE CHARACTERISTICS OF FLAX GROWN ON SOIL TYPE 4A (WAITOHI SILT LOAM, WELL-DRAINED PHASE)

*Quality index = 14.8.* Seven crops were sown on this soil type. Fibre characteristics show a moderate degree of variation.

The main variations within the group are—

- (1) Number of fibres in stem (2-3).
- (2) Layering (2-3).
- (3) Fibre size (1-2).

There were no crops with very small fibres (all in groups 1 and 2), no crops with poor fibres (all in group 1), and all crops showed good fibre shape (1-2).

The best fibre picture is given by 120B and 127, both of which were grown towards the west of the district under a more uniformly humid climate. These crops were very even throughout, although crop 127 was rejected because its average length was less than 20 in. One of the worst was 121. The main difference between crop 121 and the rest was in the waterlogging of the field when the crop was in the seedling stage. It is possible that this may have had a bearing on the final fibre picture. Of the total area, 14 per cent. was sown on soil type 4A.

Date of sowing did not appear to have much effect on these crops.

Taking the group as a whole, the crops were uniform. The fibre size was generally good, and there was only a slight tendency towards rounded fibres. The soil may therefore be regarded as moderately suitable for flax-growing.

(5) FIBRE CHARACTERISTICS OF FIBRE GROWN ON SOIL TYPE 4B (WAITOHI SILT LOAM, POORLY-DRAINED PHASE)

*Quality index* = 18. Eleven crops were grown on soil type 4B. All crops showed a strikingly wide range of variation, which may be summarized as follows:—

- (1) The greatest range of variation was in the proportion of poor fibres, which ranged from 1-4.
- (2) Fibre size, wall thickness, fibre shape, and number of fibres in the stem all showed a range of from 1-3.
- (3) Layering and bundle shape showed a moderate range of variation, only 2-3 and 3-4 respectively.

The worst crop was 135 (fibre index number = 25), which was sown late (the latest of the group, 11th November), in a very poor seed-bed cultivated when the field was too wet.

The following crops were also below average: 96 [22], 108 [20], 123 [18], Crops 123 and 118 were rejected because their average length was less than 20 in.

The better crops—99 [16], 100 [15], and 132 [15]—were sown on a slightly undulating phase of the type, which therefore approached soil type 4A in drainage properties. The effect of this variation is shown clearly in the fibre picture.

All crops, with the exception of 135, were sown about the same time.

The soil type, as a whole, may be regarded as a problem one, and the above observations would suggest that it is better avoided for linen flax. Of the total area sown in Geraldine County, 30 per cent. was on type 4B.

(6) FIBRE CHARACTERISTICS OF FLAX GROWN ON SOIL TYPE 8A  
(TAIKO SILT LOAM)

*Quality index* = 15.7. Flax grown on soil type 8A shows a fair degree of uniformity in fibre quality. The principal variation is in wall thickness of the fibre (1-3). Other fibre characteristics vary only slightly (2-3) for all fibre features except the percentage of poor fibres, which ranges from 1-2.

The worst crops were: 124 [18], 95 [18], 101 [17]. Paddock 124 was known to have had a poor tilth, which may have affected fibre quality. Crop 95 was rejected because its average length was less than 20 in.

The best crops were: 107 [14], 106 [14], 131A [14]. Of these, crops 107 and 106 lay to the west of the Geraldine district, where humidities are higher, and the soils are therefore transitional to soil type 8c, on which crops have a fibre index number of 13.0.

All crops on soil type 8A were sown about the same time, with the exception of crop 131A, which was sown early.

Crops sown on soil type 8A, which may be regarded as a moderately good flax-growing soil, represented about 22 per cent. of the total area sown.

**(7) FIBRE CHARACTERISTICS OF FLAX GROWN ON SOIL TYPE 8C  
(TAIKO SILT LOAM, LEACHED PHASE)**

*Quality index = 13.0.* Flax grown on soil type 8C shows a good degree of uniformity. The principal differences are in fibre size, number of fibres in the whole stem, and layering, which range from 1-3. The remaining characteristics show only slight variation. A good feature of this group is the low proportion of poor fibres.

**NOTE.**—The quality index is derived from considering all the crops sown on the type. However, some of the crops were sown very late, and should probably be regarded as not truly indicative of the normal fibre characteristics of the group. The quality index, therefore, should probably be rather lower—i.e., better.

The effect of late sowing shows up clearly. In the following list the crops are arranged in order of date of sowing : —

Date of Sowing.*					Crop No.	Fibre Index No
5	..	..	..	..	114	10
5	..	..	..	..	137A	13
5	..	..	..	..	137B	13
6	..	..	..	..	122	14
6	..	..	..	..	109	15

\* Date of Sowing : 5, to 14th November; 6, 15th to 30th November.

Crops 137 A, B, and 114 were situated close together, and their pictures might have been expected to agree more closely. The land for crops 137A and B, however, ploughed out of old brown-top pastures, were in exceptionally poor tilth, which might account for their poorer fibre picture.

This soil may be regarded as very suitable for linen-flax growing.

**PART IV.—CLASSIFICATION OF SOIL TYPES FOR LINEN-FLAX  
GROWING**

According to their suitability for linen-flax growing the soil types considered above may be divided into three groups.

**GROUP 1 : GOOD FLAX-GROWING SOILS**

These possess optimum drainage, neither droughty nor too wet, and have an even climate during the growing season. Group 1 includes the following soil types :—

Orari silt loam ; soil type 3 :

Taiko silt loam (leached phase) ; soil type 8C.

The best flax of the 1943 season was grown on these two soils. This flax was even and of uniformly good quality. Of the flax grown in Geraldine district, 16 per cent. was sown on soils of this group.

## GROUP 2: MEDIUM FLAX-GROWING SOILS

These soils possess some disability which may affect the crops in either a dry or a wet season *e.g.*, over-drainage (drought) or under-drainage. Group 2 includes the following soil types:—

Waitohi silt loam (well-drained phase); soil type 4A.

Taiko silt loam; soil type 8A.

They grow fairly even flax, but not of such high quality as the soils in group 1. Of the Geraldine flax, 36 per cent. was sown on soils of this group.

## GROUP 3: SOIL UNSUITABLE FOR FLAX-GROWING

Group 3 includes soils too droughty or too wet to grow flax successfully in any but an exceptional season. It includes the following:—

Orari silt loam (sandy phase); soil type 3B.

Orari silt loam (moderately shallow phase); soil type 3C.

Waitohi silt loam (poorly drained phase); soil type 4B.

Type 3B gave a very poor fibre picture and a very poor crop with many patches of false browning.

Type 3C, in spite of its average fibre index of 16.4, is included in this category, on account of the fibre variations within the group as well as the fact that late sowing is likely to lead to a very poor crop. It is inclined to be droughty, so that any projection of the growing period into the dry January-February period is likely to lead to premature dying off of the flax. Moreover, yields from the experimental sheaves from which stems for sectioning were selected were rather lower than the microscopic picture would suggest. The value of this soil for linen-flax growing, therefore, needs further study. On it false browning was common not only in the Geraldine district, but elsewhere in South Canterbury, especially in the Fairlie district, where summer temperatures are commonly slightly higher than on the coastal downlands. On the other hand, in a wet season 3C would be expected to produce moderately good flax. It drains freely and is not readily waterlogged.

On undrained fields of type 4B, late sowings are to be expected and therefore poor flax. Structure of the topsoil is poor, and unless the moisture content of the soil is exactly right it may be difficult to get a satisfactory seed-bed. When wet, the topsoil cakes together in lumps, and when dry, breaks up readily to a fine powder, which may set like cement after a shower of rain. If mole drains were drawn it should be possible to work fields rather earlier in the spring; but the structural breakdown, the consequence of constant wheat-growing over many years, will not be easily remedied, and is a problem needing immediate attention.

On the whole, if better soils were available soils included in group 3 would be better avoided for the growing of linen flax. Soils of groups 1 and 2 should be given preference.

Of the area, 48 per cent. was sown on soils of group 3.

The preceding analysis applies only to the 1943-44 season, which was moderately dry and followed the course of four seasons out of five—that is, by January the stonier soils had dried out enough to kill off weeds. The conclusions reached from considering the relationship of flax fibre to soil type and agricultural environment therefore apply only to such a season. In a wet season such as South Canterbury has once in six to eight years, the response would be totally different. Flax could then be grown on the stony and sandy soils with reasonable success.

In interpreting results it should be borne in mind that such a season as 1943-44 cannot be expected every year, and a long-term policy of flax-growing should take into account the incidence of abnormally wet seasons when the heavier soils are likely to be waterlogged but the droughty soils would grow moderately good flax.

The results of the fibre analysis are summarized in the following table :--

TABLE XVI

Percentage of Area grown on each Soil Type.	Number of Growers.	Soil Type.	Fibre Index.	Soil Rating for Linen Flax.	Remarks.
4 ..	2	3	10.5	1	Best flax grown on this type. Good fibre of uniform quality. Crops sown early.
1 ..	1	3D	24.0	3	Only one grower—very poor crop with false browning, flax uneven.
17 ..	6	3C	16.4	3	Crops show fair variation. Soil droughty and late-sown crops likely to be poor. False browning. Some crops sown early.
14 ..	7	4A	14.8	2	Moderately good fairly uniform fibre. Two crops sown early.
30 ..	11	4B	18.0	3	Very variable flax. Two rejected crops on this type. Fibre in general poor. Unsuitable for flax.
22 ..	7	8A	15.7	2	Fairly uniform fibre. Poor crops on this type are due to factors other than soil type. No crops sown early.
12 ..	6	8C	13.0	1	Good fibres of uniform quality. Variations due to differences in time of sowing, or poor seed-bed. No crops sown early.

## PART V.—RELATIONSHIP BETWEEN FIELD BACKGROUND AND FIBRE CHARACTERISTICS

Within the groups of crops sown on each soil type an attempt was made to assess the effect of such variations in the field background as date of sowing, tilth of seed-bed, previous agricultural history of the field, and climate. Since these variations were difficult to define exactly under field conditions they were only considered when they were manifested to an extreme degree—that is, tilth was considered only when it was clearly very poor, when the field had been ploughed wet and the seed-bed was hard and lumpy, or when a brown-top turf had been only partly broken up and still remained as large lumps.

### DATE OF SOWING

Late sowing appears to lead to poor-quality flax. Except in the immediate neighbourhood of the ranges, soils dry out badly in mid-January, and any crops still making growth after this period are likely to suffer badly through drought. Linen flax is intolerant of deficient moisture in the latter half of its growing season, and any circumstance which speeds up soil drought spoils the fibre.

The date of sowing is controlled to some extent by the soil type. Those with poor internal and surface drainage cannot be sown as early as those with better drainage properties. Thus the earliest sown crops were on soil type 3, which is also in other respects the best linen-flax-growing soil.

During the 1943 season, flax sown after the middle of November was, on the whole, of poor quality.

#### CULTIVATION

Crops 124, 135, and 137A and B were sown on conspicuously poor seed-beds. Crop 135 showed a decidedly poorer fibre picture than the other crops sown on the same soil type, and 124 was one of the two poorest on its soil type. Crops, 137A and B, though not the poorest in their group, were poorer than the adjacent crop 114, which was sown about the same date on the same soil type.

#### CLIMATE

Climate has been discussed earlier. Linen flax benefits from an even supply of moisture, which may be supplied by light showers during the period from mid-December to within a week or so of date of pulling, by irrigation, or by growing on a soil with maximum moisture-holding properties. By early sowing the crops are standing for as short a time as possible in the dry January–February–March period.

This effect of climate is well revealed by the fact that flax grown on soil type 8c is superior to flax grown on soil type 8A. Soil type 8A is much less leached, has a better plant nutrient status, but better flax was grown on 8c. The climate where soil type 8c occurs is, however, much more humid than that of 8A, with summer the period of constant cloud and well-scattered light rain. Nutrient status, therefore, within these limits, would seem to be less important than an even moisture supply.

#### AGRICULTURAL HISTORY OF THE FIELD

Only one crop in the Geraldine district was sown on a field of which one part had had a different agricultural history from the other, but insufficient samples were taken to pursue the difference adequately. Over the remainder of the crops there was too much uniformity of agricultural history to throw any light on this question.

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## HORMONE-TYPE WEED-KILLERS

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### Summary

Three commercial weed-killer preparations of phenoxyacetic acid derivatives have been tried out on weeds in pastures and waste places.

In all cases the grasses, except germinating seedlings, were unaffected.

Californian thistle can be considerably checked in pasture, and it appears likely that it can be killed out by successive sprays.

Convolvulus is susceptible, and the chemicals may well prove useful in eradicating this weed where it is not growing over susceptible plants.

A discussion on the properties and possible uses of the weed-killers is given.

Further work on weedicide effects of these hormone-type weed-killers is being carried out.

### INTRODUCTION

It has been known for some time that plant hormones and other plant growth substances, when applied to plants in strengths greater than optimum, cause deformation and death of plant tissues. Plants vary in their responses to these substances, and this led to the idea that such compounds might prove useful as selective weed-killers.

Work along these lines has been carried out in England and America. In England the work was concerned primarily with the control of annual weeds in cereal and other crops. Of the chemicals tried, 2:4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid were found to be the most effective(1, 2), the latter being most favoured in England(3). This substance was found to kill such weeds as charlock, *Ranunculus arvensis*, *Thlaspi arvense*, and others when sprayed at the rate of 1 lb. of the sodium salt per acre. The cereal crop remained unharmed(1).

In America, following the finding by Zimmerman and Hitchcock(4) that 2:4-dichlorophenoxyacetic acid (2,4-D) was a plant-growth substance, Marth and Mitchell(6), Hamner and Tukey(7), and others experimented with this compound as a selective weed-killer. The acid is not soluble in water, but Mitchell and Hamner(5) had found the polyethylene glycol preparation, "Carbowax 1500," to be a suitable carrier, having the added advantage of somewhat increasing the effects of the acid on plants. In much of the work done, "Carbowax 1500" has been used as the carrier, though other commercial preparations are now available.

The work has been taken up by the Greens Section of the United States Golf Association(8, 9). The rate of application recommended was 200 American gallons per acre of a solution containing 0.1 per cent. of the acid (2,4-D). Using this strength, a large number of troublesome turf weeds could be killed without in general harming the grasses. The bent grasses were somewhat sensitive, especially if kept cut very short, but in an instance when all the top growth was killed the plants later recovered fully.

To date, trials using the weed-killers in the form of dusts have indicated that more of the active compounds are needed than when applied as sprays(1, 10). There is also greater danger of drift harming susceptible plants some distance from the area treated.

The use of 2,4-D to control germinating weed seeds in composts has recently been suggested(11).

It has been shown that 2,4-D causes abnormal cell division and growth in plants(12), with a depletion of reserves of starch and soluble carbohydrates(13).

It is of interest that iso-propyl phenylcarbamate has also been found to be a selective weed-killer, but its effects are the reverse of those of the phenoxy compounds, being toxic to cereals at concentrations which do not harm such plants as sugar-beet and charlock(14).

#### MATERIALS AVAILABLE

*Dow Weed-killer.*—This is apparently a mixture of 2:4-dichlorophenoxyacetic acid and sodium bicarbonate. The powder dissolves in warm water with effervescence. It contains about 70 per cent. of the active chemical.

*Methorone (10 per cent. Solution).*—This is a solution of the sodium salt of 4-chloro-2-methylphenoxyacetic acid.

*Weedone.*—2:4-dichlorophenoxyacetic acid, 9.6 per cent. by weight (about 9.2 per cent. W/V), dissolved in some emulsifying oil.

*Methorone Dust.*—1 per cent. of 4-chloro-2-methylphenoxyacetic acid with china clay diluent.

#### EXPERIMENTAL

##### *Persistence in Soil*

Unsterilized soil in two seed-boxes (22 in.  $\times$  11 in.  $\times$  2 $\frac{3}{4}$  in. high) was sprayed on 9th August, 1945, with 34 ml. (box A) and 77 ml. (box B) of a solution of 0.714 per cent. of Dow weed-killer. This is equivalent to about 10 lb. and 22 lb. respectively of active chemical per acre. These strengths are several times greater than the strongest used in field trials. The soil in each box was thoroughly mixed, and then sown at intervals between 20th August and 24th September, 1945, with radish-seed, this plant being very sensitive to the chemical. In box A one plant of the sowing on 28th August, though affected, recovered sufficiently to bulb, but it was not until the sowing on 11th September that the plants appeared normal. In box B normal germination and growth did not occur before the row sown on 19th September. Of the earlier sowings, many seeds failed to germinate at all. Some germinated to form very abnormal plants with swollen hypocotyls and other abnormalities, a few coming up upside down. A few germinated about a month after sowing, to form apparently normal plants. Some grass-seeds (probably rye-grass) germinated early in the trial in both boxes, but soon died. Towards the end of the trial several grass seedlings, as well as clover and other weeds, came up and were growing normally. Some worms remained alive and healthy throughout the experiment.

##### *Californian Thistle (Cirsium arvense)*

*Preliminary Trial.*—On the 18th October, 1945, two plots each of 1 square rod were marked off in a patch of Californian thistle growing in pasture and were sprayed with Dow weed-killer at strengths of 0.071 per cent. and 0.286 per cent. of powder respectively, using 1 $\frac{1}{2}$  gallons per plot and a rather coarse nozzle, the applications being equivalent to 1 lb. and 4 lb. respectively of active chemical per acre. Other species present were rye-grass (*Lolium*), white clover (*Trifolium repens*), daisies (*Bellis perennis*), catsear (*Hypochoeris radicata*), *Plantago lanceolata*, cocksfoot (*Dactylis glomerata*), Yorkshire fog (*Holcus lanatus*), and other grasses.

A fortnight later the thistles were much affected, especially with the stronger spraying, the leaves being much deformed and yellowed. In the stronger plot the daisies were yellowed and sickly looking and the plantain much deformed.

A month after spraying, the thistles in the weaker spraying were further deformed, with the bases of the stems abnormally thickened and the tops yellowed and without flowers. Some shoots had died. In the stronger plot nearly all the shoots were dead or dying. *Plantago lanceolata* was largely killed out and daisies much reduced. The grasses were unaffected. The clover was still present, though somewhat reduced in quantity. The catsear was not greatly affected.

In the weaker plot, two months after spraying, many tops of the thistles were dead and others swollen at the base and yellowed. Regeneration was starting in places distant from the old shoots, the shoots being smaller and weaker than the original ones. In the stronger spraying all the original tops were dead, but some regeneration was occurring. The catsear plants had a number of peculiarly lobed and forked leaves.

The plot with stronger spray was sprayed again on 6th February, 1946, using 1.1 gallons of 0.25 per cent. Dow weed-killer (equivalent to 3.1 lb. of active chemical per acre) and a finer nozzle.

Two and a half months later only three very weak shoots of Californian thistle were found in the plot. There was a complete kill of daisy, catsear, and *Plantago lanceolata*. Two small plants of *Ranunculus repens* (probably seedlings) were seen. Clover was reduced considerably, but still present throughout. The grasses were unaffected.

*Second Trial.*—On the 4th December, 1945, plots of 1 square rod were laid out in an area of pasture carrying a dense stand of Californian thistle. Each plot was sprayed with 1.1 gallons of liquid, using a fine nozzle. The following sprayings were carried out:—

Plot.	Strength of Spray Solution.	Equivalent Quantity of Active Chemical per Acre.
		lb.
A	0.125 per cent. of Dow weed-killer .. .. .	1.55
B	0.125 per cent. of Dow weed-killer plus 0.025 per cent. of aerosol T (10 per cent. solution) to act as a wetting and spreading agent	1.55
C	0.25 per cent. of Dow weed-killer .. .. .	3.1
D	0.5 per cent. of Dow weed-killer .. .. .	6.2
E	0.1 per cent. of Methoxone .. .. .	1.75
F	0.2 per cent. of Methoxone .. .. .	3.52

The pasture was predominantly rye-grass and white clover with a few other grasses present. At the time of spraying the thistles were at the stage of early bud development.

On the 18th December a further plot was sprayed with Weedone at a strength of 1 part of Weedone to 60 parts of water (equivalent to 2.7 lb. of active chemical per acre).

On the 11th December all the tops of the thistles were bent over. In the Methoxone plots there was a burning effect on the leaves, turning them a grey colour.

Five weeks after spraying most of the shoots of the thistles in all plots were dead, though a few were still green at the base. There was not much difference between the plots, and the aerosol showed no noticeable effect. The most complete kill of the tops was in plot D. Clover was still present in all the plots, though reduced in quantity in the stronger sprayings. Regeneration of the thistles started a few weeks later, small shoots appearing in all plots, but rather more showing in the weaker sprayings.

All plots were sprayed again on the 20th February, 1946, using the same strengths of solutions as before. All the young shoots were killed within three weeks.

At the beginning of March the soil was examined for live roots. It was found that most of the original vertical shoots were completely killed. Some of the horizontal roots still remained, however, though, judging from the number present in adjacent unsprayed areas, many had been killed and had largely rotted away.

By 9th April regeneration had again occurred in all plots, though rather sparsely in plot D. Clover was still present, but further reduced in quantity.

The plots were sprayed for the third time on 17th April, 1946. At the same time a preliminary trial of Methoxone dust was started, a plot of half a square rod being dusted with 10 oz. of the 1 per cent. dust (equivalent to 2 lb. of Methoxone per acre). The thistles in this plot were at the rosette stage, the earlier growth having matured and died down.

A fortnight later all the leaves of the thistles in the dust and spray plots were yellowed and distorted. Some shoots had been eaten off by sheep. No new shoots appeared before the winter.

#### *Convolvulus and other Weeds*

*First Trial.*—On 25th October, 1945, a trial spraying was carried out on some convolvulus (*Calystegia sepium*), buttercup (*Ranunculus repens*), wild turnip (*Brassica campestris*), and other weeds in rough grass, using about 2 gallons of 0.07 per cent. Dow weed-killer per square rod (equivalent to 1.6 lb. of 2,4-D per acre) and a coarse nozzle. Owing to the coarseness of the spray, some of the plants did not receive much of the solution, especially those partly protected by the grass. The main grasses were cocksfoot and prairie-grass (*Bromus catharticus*), and other plants present were couch-grass (*Agropyron repens*), docks (*Rumex* spp.), periwinkle (*Vinca major*), and cleavers (*Galium aparine*).

One week after spraying, the convolvulus and docks were greatly curled up and distorted, and buttercup leaves twisted and discoloured. The leaf bases of the wild turnip were greatly swollen and the growing points appeared to have aborted, though at that stage the leaves were still green.

Two weeks after spraying, the wild turnip and top growth of convolvulus were mainly dying, though there was some recovery, probably due to incomplete cover by the spray. Many of the docks appeared to be recovering, the periwinkle and cleavers had completely recovered from the very slight distortions they had shown, and the grasses showed no effects. The spray used appears to have been too weak.

*Second Trial.*—On 19th November, 1945, comparative sprayings were made with 0.5 per cent. Dow weed-killer and 0.4 per cent. Methoxone on an area of convolvulus, buttercups, and wild turnips with the rough grass removed, and also on convolvulus, Californian thistle, and periwinkle growing in rough grass. A fine nozzle was used and the solutions applied at about  $1\frac{1}{2}$  gallons per square rod. This is equivalent to an application of about 7 lb. of 2,4-D and 8 lb. of Methoxone per acre.

The Methoxone had a quicker initial effect, causing grey or bleached patches in the leaves of Californian thistle and wild turnip. However, the final effects of the two sprays appeared much the same. Wild turnip and all the above-ground parts of the convolvulus were killed and the buttercup much checked. Later it was found that all the rootstocks of the buttercup were rotten. Sheep let into the rough grass area about a fortnight after spraying ate all the sprayed shoots of Californian thistle and convolvulus, and also one large dock which was in one of the areas sprayed.

The periwinkle was hardly checked at all in growth. Some self-sown potatoes received a temporary check, but recovered and produced a crop of tubers.

On later digging up the convolvulus the greater proportion of the rhizomes were found to have been killed, but live pieces remained at places distant from the sprayed shoots, and these were capable (in both sprayings) of regenerating shoots when placed in sand.

*Third Trial.*—On 22nd February, 1946, convolvulus along a fence-line was sprayed with Methoxone 0.1 per cent. and 0.2 per cent., Dow weed-killer 0.125 per cent. and 0.25 per cent., and Weedone 1 part to 100 parts of water and 1 part to 60 parts, sufficient spray being used in each case to cover the foliage. The convolvulus at this stage had completed most of its active growth and was in full flower.

Deformations first appeared with the Methoxone sprayings, but within a month the top growth was dead in all treatments. No new shoots had appeared by the end of May. However, live rhizomes were found when the ground along the fence-line was dug.

*Convolvulus in Shrubs.* In order to test whether convolvulus could be killed when growing in shrubs without killing the shrubs, several shrubs well covered with convolvulus were sprayed on 20th December, 1945, with 0.5 per cent. of Dow weed-killer and with 0.4 per cent. Methoxone.

All the top growth of the convolvulus was killed and no regeneration occurred within four months, but during May a few weak shoots appeared, mainly towards the edge of the sprayed areas. Of the shrubs, broadleaf (*Griselinia littoralis*) and *Escallonia* were unaffected. The shoots of a species of *Buddleia* which had been sprayed were killed, so the plant might have died had the whole of it been sprayed. The outer twigs of *Lonicera nitida*, *Myrtus obcordata*, and a flowering currant (*Ribes sanguineum*) were killed, but by April the plants were shooting again from below.

*Dust Application.*—On 17th April, 1946, Methoxone dust was applied to convolvulus growing in a fence-line and to adjacent ground on which was growing *Plantago major*, dandelions, cleavers, *Barbarea* sp. (cress), young plants of *Ranunculus repens*, many seedling docks, and white clover, as well as a number of grasses. The application was made with a hand duster at a rate equivalent to about 2 lb. of Methoxone per acre, and there was no wind to cause drift of the dust. All species except the convolvulus were growing actively at the time of application.

The top growth of the convolvulus was soon yellowed and was all dead within three weeks. A month after spraying, all seedling docks and plants of *Plantago major* were dead dandelion and *Ranunculus repens* mostly dead. Most of the old leaves of the cress were killed, while some young leaves which had appeared, subsequently died. The clover was little affected, and the cleavers and grasses quite unharmed.

*Nasturtium palustre*.—This cress is a perennial and shoots persistently from the root when hoed off, being most difficult to eradicate. On 24th October, 1945, a preliminary trial was made on a small area using a spray of 0.07 per cent. of Dow weed-killer. All the terminal shoots and most of the leaves were killed, but within six weeks healthy shoots had again appeared from the lower parts of the stems and the roots.

On 9th November, 1945, a further spraying trial was made on another area using Dow weed-killer of strengths from 0.125 per cent. to 0.5 per cent. with and without aerosol.

Within three weeks all the terminal shoots were killed in all plots and all the leaves also in the stronger sprayings, though in all plots some of the bases of the stems remained green. Aerosol had no noticeable effect. No new shoots appeared within two months, but after this the plants in all plots began to shoot again.

On 21st February, 1946, all plants were sprayed again, this time with 0.4 per cent. Methoxone. All above-ground parts of the plants were killed and no regeneration had occurred by the end of May. Thus it seemed probable that the plants were then dead, as they normally remain leafy throughout the winter.

In another area, which was sprayed on 18th December with 0.5 per cent. of Dow weed-killer after some seed had set, all the leaves and buds were killed but the stems remained green, and that seed which had set appeared to be ripening normally when the tops were pulled up and burnt.

*Blackberry (Rubus fruticosus var.)*.—On 20th January, 1946, two bushes were selected, one being sprayed with 0.5 per cent. Dow weed-killer and the other slashed back with the idea of later spraying the young shoots.

One month later the sprayed bush was not much affected, except for a few young shoots which had been killed at the tips and were much deformed. The slashed bush had grown new shoots 3 in. to 1 ft. in length, each with several fully expanded leaves. Both bushes were sprayed again with 0.5 per cent. Dow weed-killer.

Six weeks later the plants were again examined. Most of the leaves had been killed on the bush not slashed, but the stems were still alive. On the other bush all the young shoots had been killed, though a few others had later appeared. However, two roots were pulled up, and it was found that, although the plants had a few young growing shoots, the main root had in each case died and was rotting, only some fine lateral roots still being alive.

At the end of May the tops of most of the stems of the bush not slashed were dead, but the bases of the stems were all still alive and several new shoots were appearing. More than half the roots of the slashed bush were dead, but there were a few healthy shoots on the other roots. Thus a single spraying of the slashed bush had had considerably more effect than two sprayings of the one not slashed.

*Other Weeds.*—A small patch of bracken (*Pteridium esculentum*) and one plant of hard fern (*Paesia scaberula*) were sprayed on the 20th January, 1946, with 0.5 per cent. Dow weed-killer. In both plants the fully mature leaves were unaffected, but the immature leaves of both were considerably twisted a fortnight after spraying, and in the case of hard fern all the growing rhizomes were killed. No new fronds had appeared on either plant by the end of May.

A bulb-forming species of *Oralis* was sprayed with a solution of 0.5 per cent. of Dow weed-killer, but the bulbs did not appear to be greatly affected and a new crop of leaves appeared within six weeks.

### DISCUSSION

From reports and tests to date, these new weed-killers have been shown to have several useful qualities.

They are very selective in action, killing many plants completely, while others, notably grasses, are quite unharmed.

They are comparatively slow in action, and this, added to the fact that the substances are conducted considerable distances through plants(15), leads to their killing the underground parts of some perennial weeds as well as the tops(7).

The trial here confirmed the finding of Nutman, Thornton, and Quastel(2) that the toxicity of 2 : 4-dichlorophenoxyacetic acid is destroyed in less than two months in unsterilized soil, even when a very high rate of application is used. The compounds are thus unlikely to poison the soil.

The compounds are non-corrosive to spray equipment. However, thorough washing is needed to remove all residues from the equipment if it is required for other purposes.

The compounds are apparently non-toxic to men and animals, and probably also to soil micro-organisms. Stevenson and Mitchell(16) found that 0.02 per cent. of 2,4-D checked, and 0.08 per cent. prevented, growth of various bacteria, but such concentrations would not occur in the soil solution after spraying at strengths now used. No effects were observed on two fungi which were tested. The fact that the underground parts of plants killed by the compounds are quickly decomposed indicates that soil micro-organisms are probably not adversely affected by them.

According to most reports, the effectiveness of the sprays is not reduced by rain soon after application, especially if Carbowax is used as a carrier(8). However, in one report(17) it is stated that heavy rain within twenty-four hours considerably reduces the effectiveness of aqueous sprays. No definite conclusion has yet been obtained here on this point.

The acids themselves are not soluble in water, and for sprays they need to be made soluble, either by use in the form of soluble salts (the sodium, potassium, ammonium, and calcium salts are soluble) or by means of a water-soluble or emulsifying carrier. So far no definite differences in final effectiveness have been apparent between the preparations tried, though Methoxone shows initial effects more quickly with some burning of the foliage. For practical purposes the main factor determining which preparations are best will be the retail price in New Zealand, and little information on this is yet available. Carbowax is stated to increase the effectiveness of 2,4-D, but it will also considerably increase the price of the product.

In general, plants are more susceptible to the compounds when they are growing actively. A higher temperature at and after the time of application usually results in a quicker and more complete kill(7, 8). Marth and Davis(18) studied the role of temperature and found, using several weeds common in temperate climates, that quickest death occurred when the temperature was between about 65° F. and 80° F. Plants kept at a temperature between 32° F. and 40° F., where there was little or no growth, were much slower to show effects when sprayed, apparently surviving in many cases. However, if they were later subjected to higher temperatures, which should normally cause renewed growth, they showed the typical distortions due to the weed-killer and soon died. A field trial on *Plantago lanceolata* confirmed this finding—plants sprayed during the winter when the ground was frozen died the following spring. It appears from this that the plants absorb the chemical at the lower temperature and retain it in their tissues in sufficient quantities to cause death when active metabolism is again possible.

Applications of the weed-killers are best made at warm temperatures when growth is vigorous and the soil moist. However, it is important that the plants, to be encouraged, should be at a stage where they can quickly fill the gaps caused by the death of the weeds, thus not allowing further weed seeds to germinate.

Tukey, Hamner, and Imhofe(12), working with *Convolvulus arvensis* and *Sonchus arvensis*, found that 2,4-D stimulated the plants to abnormal cell division and growth in the stems, rhizomes, and roots, such growth causing the disorganization of the tissues. Reserves of starch and other available carbohydrates were greatly depleted in all the storage tissues of the plants(12, 13). This depletion of the food reserves and the disorganization of the metabolism of the plants is presumably the cause of death. Depletion of food reserves is an important factor in the control of weeds. In the cases where the weeds sprayed, though affected, are able to recover, this depletion of reserves must result in their being weakened and thus more rapidly killed by further applications.

From trials here it does not appear possible to kill out Californian thistle growing in pasture by a single application of any of the preparations tried. However, the remaining roots must be weakened by the treatment, and several successive applications should result in the eradication of the weed. There is some New Zealand evidence to suggest that it is easier to kill Californian thistle in ploughed areas, where the roots are cut into shorter lengths, provided that all roots are bearing above-ground shoots at the time of application.

*Nasturtium palustre* and *Calystegia sepium* appear to be easier to kill, but with these also repeat sprays may be necessary for complete eradication where the plants are well established. It may be possible to eradicate convolvulus when growing over shrubs without permanently harming the shrubs. The sprays used were about four times stronger than needed to kill all the top growth of the convolvulus. It is likely that some shrubs will prove to be too sensitive for such treatment.

Many woody plants are checked but not normally killed by weed-killers. Among these are included several noxious weeds, which readily shoot again from the roots when cut back. It may be possible to control such weeds by first stimulating them to active growth by cutting back or burning off the



top growth, and then spraying the young shoots as soon as they have sufficient leaf surface to absorb the spray. Blackberry shows promise of control by this means, though further trials are needed.

Dusts have the advantage over sprays of being lighter to carry. Also, they can be applied through the equipment used for distributing fertilizers, which is normally already available on farms. However, it is not possible to distribute them as evenly as sprays, and also they do not readily adhere to the foliage unless it is damp. However, the addition of hygroscopic agents as suggested by Marth, Davis, and Mitchell(10) may help in getting over this latter difficulty, and should decrease to some extent the danger of drift. Further trials are necessary before the general applicability of dusts can be determined.

Hamner, Moulton, and Tukey(11) suggest that the weed-killers may be useful in the control of germinating weed seeds in composts. In trials it was found that treatment at the rate of 10 parts of 2,4-D per 1,000,000 of compost was sufficient to prevent the germination of most weed seeds.

Apart from the bents, all grasses so far tried have been found to be resistant. The bent grasses are somewhat susceptible, but capable of complete recovery. All members of the Cruciferae so far tried have been found susceptible, most of them being readily killed. Apart from these cases, there is little correlation between the botanical relationships of plants and their susceptibility to the compounds.

The following list of weeds commonly occurring in New Zealand have been found in trials to be killed by Methoxone or some 2,4-D preparation, those marked \* being from overseas reports: *Ranunculus repens*, *Brassica campestris* (wild turnip), \**Sinapsis arvensis* (charlock), *Capsella bursa-pastoris* (shepherd's purse), *Barbarea* sp. (cress), \**Lepidium virginicum* (pepper-grass), \**Erophila vulgaris* (Whitlow grass), *Coronopus didymus* (hog-cress), \**Amaranthus retroflexus* (redroot), \**Amaranthus* spp., \**Malva rotundifolia* (dwarf mallow), \**Potentilla* sp. (cinquefoil), *Lupinus arboreus*—young plants, *Daucus carota* (wild carrot), *Conium maculatum* (hemlock), *Foeniculum vulgare* (fennel), \**Hydrocotyle* spp., *Bellis perennis* (daisy), \**Erigeron canadensis* (Canadian fleabane), \**Artemisia* sp. (wormwood), \**Arctium* sp. (burdock), \**Centaurea solstitialis* (Yellow star thistle), *Carduus nutans* (nodding thistle), *Carduus tenuiflorus* (winged thistle), *Cirsium lanceolatum* (Scotch thistle), *Hypochoeris radicata* (catsear), *Taraxacum officinale* (dandelion), \**Sonchus arvensis* (perennial sow thistle), *Sonchus oleraceus* (sow thistle), *Plantago major* (greater plantain), *Plantago lanceolata* (rib-grass), \**Convolvulus arvensis*, *Calystegia sepium*, *Calystegia tugoriorum*, \**Dichondra repens*, \**Veronica tournefortii*, \**Veronica arvensis* (field speedwell), *Prunella vulgaris* (self heal), and \**Lamium amplexicaule* (henbit).

Plants which, though affected, are normally able to recover from one application, include: *Rumex acetosella* (sheep sorrel), *Rumex* spp. (docks), \**Polygonum persicaria* (lady's thumb), \**Spergula arvensis* (spurrey), *Nasturtium palustre*, *Achillea millefolium* (yarrow), \**Chrysanthemum leucanthemum* (ox-eye daisy), *Cirsium arvense* (Californian thistle), \**Portulaca oleracea* (purslane), and *Trifolium repens*.

Resistant species include the grasses, *Vinca major* (periwinkle), *Galium aparine* (cleavers), *Viola* spp., *Solanum tuberosum* (potato), and others.

There is disagreement between different workers in some cases. For instance, *Chenopodium album*, *Polygonum aviculare*, and *Oxalis* spp. are given by some workers as being resistant, and others as being susceptible. *Stellaria media* (chickweed) and *Cerastium* sp. (mouse-eared chickweed) have been reported(8) as being susceptible, but in trials here they have been found resistant.

Woody plants are in general more resistant than herbs, but some such as *Rubus* spp., Willows (*Salix* spp.) and *Robinia pseudoacacia* have been found overseas to be killed by the sprays. Young trees are more sensitive than older ones of the same species.

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## STUDIES ON SOME NEW ZEALAND ROMNEY MARSH STUD FLOCKS

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### PART I.—GENERAL INTRODUCTION

#### *Summary*

A general description of the stud flocks (size and management) and the stud farms (meteorological data, soils, and pastures) on which most of these studies are based is given.

#### INTRODUCTION

THE purpose of the present studies of certain New Zealand Romney Marsh stud flocks is to present some data pertaining to reproductivity, fecundity, and productivity of stud sheep kept under commercial stud conditions. In addition, it is hoped that these studies may also be helpful in indicating the general trends in the breeding policies of Romney studs, as well as various breeding problems, and their relative claims, for further investigations. Lastly, the opportunity for scrutinizing the method of recording adopted by the Fleece-testing Department has not been overlooked.

In studies of this type it is rather difficult to decide on the method of presentation. The information on some problems is much fuller than on others, and, again, the records of some items are much more carefully kept in one year or in one stud flock than in others. Accordingly, it has been necessary on some occasions to rely chiefly on information collected from a single stud or covering one year only, and to treat some problems less fully than their actual importance merits. Consequently, some of the results are presented with more confidence than others.

#### MATERIAL AND METHODS

##### *The Size of Stud Flocks*

The study is based on two commercial stud flocks, which will be called A and B. Occasionally references will be made to stud C. Stud A consisted up to 1941 of approximately 1,500 breeding-ewes. In 1942, however, the stud was reduced to approximately 1,000 breeding-ewes. Studs B and C comprised approximately 400 and 200 breeding-ewes respectively.

It is of interest to note that flocks ranging in numbers from 201 to 500 and from 501 to 1,000 comprise respectively 22.7 per cent. and 23.6 per cent. of all the flocks in this country (*N.Z. Agr. & Pastoral Statistics*, 1943-44).

##### *Management*

The sheep were kept under conditions typical of those prevailing on the flat country in the Manawatu district. The following gives a general idea of the salient points of the stud-farm routine as observed on the above farms.

The breeding season begins in March, when the rams are put out to their respective paddocks, from which they are taken out sometime in May. Prior to the breeding season the ewes are sorted out, the unwanted ones culled, and the rest allotted, mostly on appearance, performance, and pedigree, to the rams. Studs A and B do not practise any deliberate flushing of ewes. A big proportion of ewes in stud B and some in stud C were hand-served.\* No supplementary feeding of in-lamb ewes was normally practised. The lambing season begins in August and continues till October, the lambs being docked about a fortnight after birth and tagged with brass ear-tags either at docking or shortly after birth (stud C). They are weaned usually late in December either on to special crops, with an adequate grass run-off which has been heavily grazed by cattle, or on to specially prepared grass paddocks. Generally the ram hogget (especially the "tops") are taken to dairy-farms and run there until the end of July or August, or, alternatively, the ram hoggets (and often the ewe hoggets) go on to chou moeller or swedes first and on to grass paddocks later.

The ram hoggets are shorn early in August, while the ewe hoggets are usually shorn in September or October. The fleece weights of all sheep are always recorded. The hoggets' wool is described prior to shearing and their conformation is described after shearing. At that time the ewe hoggets are also sire grouped.

#### METEOROLOGICAL DATA

The rainfall and temperature data, based on observations made over the past thirteen years by the Massey College and Plant Research Bureau meteorological station, were compiled by Fife (1945). The average rainfall was 38 in., varying from 32 in. to 51 in. The number of wet days varied from 150 to 190 per annum. The mean temperature was 55° F. and the mean maximum temperature 62° F.

#### SOILS

The following is the general description of the soil types as classified by the Soil Survey Division (Grange, 1945): Farms A and B, mostly located on "Kairanga silt loam, a recent soil derived from alluvium under a kahikatea, matai, rata forest. It occurs on flat slopes, and a common profile is 6-12 in. grey heavy silt loam on clay loam mottled grey and brown.

\* The following is the outline of the general technique: a "teaser" (a vasectomized ram) is used for finding out the ewes on heat. On the average, one teaser, which stays with the ewes all the time, is sufficient for a mob of fifty ewes. He is raddled, the colours, yellow, blue, and red, being changed every ten days. The ewes marked by the "teaser" at night or after 3-4 p.m. are brought early in the morning and again in the late afternoon to the sire to be hand-served. A very fit ram can tup three ewes within ten to fifteen minutes. Then the interval increases to from ten to thirty minutes. Sometimes a ewe, if still on heat, is tugged again in the evening and following morning. The rams during the period of hand-service, or, if out of condition, a month or two prior to it, receive about a pound per day of supplements consisting of oats, maize, bran, and linseed cake or peas.

The fertility is high to very high." The C farm is mostly located on "Tokomaru silt loam, a yellowish-grey loam derived from alluvium under a podocarp forest. It is found as gently sloping terraces." The profile has been described by Fife (1945). The fertility is moderate to high.

#### PASTURES

The pastures described by Goot (1945), in general, hold true for these farms—viz., dominant rye-grass and white-clover pastures which are top-dressed, when necessary, with superphosphate and lime at the rate of 2 cwt. of super and up to 1 ton of lime per acre.

Although the carrying capacity of these pastures is high, amounting to, say, five to six ewes per acre, this is not fully utilized, as the stud sheep are usually understocked and not more than three to four ewes per acre are carried.

#### RECORDS

The material on which these studies are based falls mainly under three headings:—

- (1) Records kept by this department for studs A and B.
- (2) Some additional private records kept by the studmaster of stud B.
- (3) Records kept by a sheep-farm manager for stud C.

The main records were kept in the form of index cards as described by Waters (1939). In addition, the mating lists, the annual lists of docked lambs, and other records kept as routine procedure proved to be of great value.

With regard to the records kept by this department, it should be pointed out that the co-operation between the breeders and the departmental staff was very close. Nearly all the information was collected in the field, the staff participating in all the major stud operations, such as selection of breeding-ewes, putting out the ewes to the rams, "boxing," docking and tagging, description of hogget wool and conformation of two-tooth, culling, shearing, &c. Other information, especially on deaths, accidents, &c., was entered into the index cards periodically as it was received from the studmasters. Such information is not always full and exact, but when sheep have disappeared from the stud without any record it has been possible, at least, to fix the time of their disappearance, and they are referred to as "missing" sheep.

The additional private records of stud B are mostly confined to hand service and occasionally to lambing dates. They were well and meticulously kept by a particularly keen and progressive studmaster.

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## PART II.—AGE COMPOSITION OF STUD SHEEP

*Summary*

(1) The age composition of sheep in studs static in numbers is analysed, and some conclusions pertaining to reproduction, breeding policy, and progeny-testing are drawn.

(2) The mean age of breeding-ewes at mating is three years four months, the mode falls within the two-tooth (one and a half years old) class, and the average productive lifetime is four years. The mean annual replacement rate is just over 28 per cent. These figures are believed to be fairly representative for studs static in numbers in this country.

(3) The mean age of rams at mating is three years two and a half months, and the mode again falls within the two-tooth group. The average lifetime is approximately four years, and the rate of replacement is approximately 30 per cent. In studs where progeny-testing is practised, the mean age and productive lifetime are lower and replacement rate is higher.

(4) Analysis of the age composition of rams in studs where progeny-testing is known to be practised, and in other studs static in numbers, revealed that the use of progeny-testing in this country is still in an experimental stage.

(5) The mode and the mean number of ewes per sire in paddock mating are 50 and 47.6 respectively. The age of rams, except hoggets, seems to be of minor importance in apportionment of ewes.

## INTRODUCTION

THE flock composition, and particularly the proportion of breeding-ewes falling into different age groups, has received little attention in studies on sheep. Apart from the significance of age, especially in reproduction and multiple birth, which has long been recognized, there is ample scope for inquiry into the various characteristics of sheep breeds and husbandry under similar and or dissimilar environmental conditions. Moreover, the knowledge of age composition of a flock would not only facilitate comparisons within and between breeds of sheep, but would also throw, as it is hoped to show later, considerable light on questions which it is convenient to group under the general terms of reproduction, production, and improvement.

## AGE OF BREEDING-EWES

In order to determine the age composition of breeding-ewes in New Zealand Romney stud flocks, the data available in the department (see Part I), were analysed. Soon, however, it became evident that not only the increase or decrease in flock numbers, but also the breeding policy and practice such as hand-service, could markedly alter the proportion of ewes falling into different age groups. As studs B and C are expanding in sheep numbers, and as in both hand-service is practised, it was decided to base the calculation on stud A only.

The data which are given in Table I show the number and the percentage of ewes according to their ages in a stud which has been stationary in numbers for several years. These data were extracted from index cards and lists of dams at mating—viz., in March of each year—and they do not include those ewes which were sold as in-lamb ewes or bought in. The number of such ewes is very small. In 1942 the stud was reduced by approximately one-third. In spite of this, the average proportion of ages as shown in the last two columns in Table I does not seem to have changed very much.

TABLE I.—AGE COMPOSITION OF BREEDING-EWES IN STUD A BEFORE MATING IN MARCH

Age.	1941.		1942.		1943		1944.		1945.		1941-45.		1942-45.	
	Number.	Per Cent.	Number	Per Cent.	Number.	Per Cent.	Number	Per Cent.	Number	Per Cent.	Number.	Per Cent.	Number.	Per Cent.
Two-tooth	465	30.14	278	26.86	335	32.59	267	27.10	295	26.92	1,640	28.84	1,175	28.35
Four-tooth	268	17.37	268	25.89	187	18.19	243	24.67	225	20.53	1,191	20.94	923	22.27
Six-tooth	317	20.55	178	17.20	189	18.38	138	14.01	211	19.25	1,033	18.16	716	17.28
Full-mouth	221	14.32	175	16.91	121	11.77	137	13.91	120	10.95	774	13.61	553	13.36
5½ years	134	8.68	91	8.79	111	10.80	90	9.14	121	11.04	547	9.62	413	9.97
6½ years	69	4.47	26	2.51	64	6.22	70	7.11	56	5.11	285	5.01	216	5.21
7½ years	48	3.11	12	1.16	16	1.56	32	3.25	42	3.83	150	2.63	102	2.46
8½ years	7	0.45	5	0.48	4	0.39	6	0.61	21	1.92	43	0.76	36	0.87
9½ years	9	0.59	..	..	1	0.10	1	0.10	3	0.27	14	0.25	5	0.12
10½ years	3	0.19	1	0.10	..	..	1	0.10	1	0.09	6	0.11	3	0.07
11½ years	2	0.13	..	..	..	..	..	..	1	0.09	3	0.05	1	0.02
12½ years	..	..	1	0.10	..	..	..	..	..	..	1	0.02	1	0.02
Total ..	1,543	100.00	1,035	100.00	1,028	100.00	985	100.00	1,096	100.00	5,687	100.00	4,144	100.00

Table II, which is based on the last column in Table I, gives the average proportion of ages per one hundred breeding-ewes in a "stationary" stud flock. Note, firstly, the gradual falling off in numbers of breeding-ewes as their ages increase (Fig. 1), and secondly, as corollary to the first, the predominance of younger ewes in the flock. In this example the stud flock consists at mating-time of over 50 per cent. of ewes not older than four-tooth (two and a half years), over 81 per cent. of ewes not older than four and a half years, and nearly 97 per cent. of ewes not older than six and a half years, on the average.

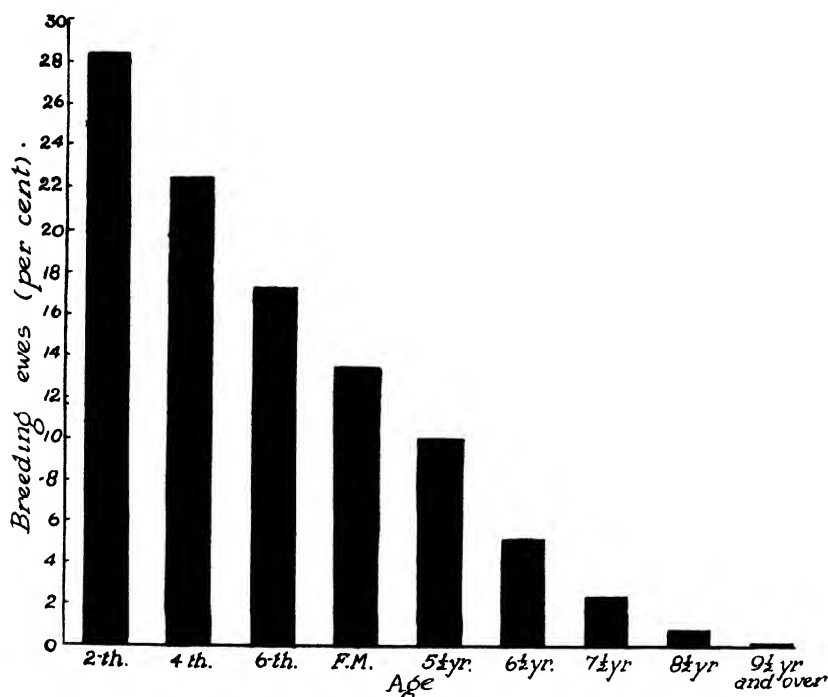


FIG. 1.—Showing percentage age-distribution of breeding-ewes.

TABLE II.—AVERAGE AGE COMPOSITION OF BREEDING-EWES IN A NEW ZEALAND ROMNEY MARSH STUD FLOCK STATIC IN NUMBERS (BASED ON STUD A)

Age.	Per 100 Ewes	
	Number.	Total.
Two-tooth .. .. .	28.4	28.4
Four-tooth .. .. .	22.3	50.7
Six-tooth .. .. .	17.3	68.0
Full-mouth .. .. .	13.4	81.4
5½ years .. .. .	10.0	91.4
6½ years .. .. .	5.2	96.6
7½ years and over .. .. .	3.4	100.0
Total .. .. .	100.0	..



The average age of breeding-ewes is three years four months at mating-time, or approximately three years nine months at lambing-time. The latter figure is lower than that given by Lush (1938)—viz., four to four and a half years.

The figures in Tables I and II have also been used to calculate the average productive life of ewes in stud A (static in numbers). This was found to be four years.

It would be of considerable interest to know how representative those figures are. At present there is no way of finding out, although some indication can be gleaned from flock-books. With this end in view, figures published annually in the "N.Z. Romney Marsh Flock-book" (1942, 1943, and 1944) were analysed, and the results summarized in Table III.

TABLE III.—PROPORTION OF TWO-TOOTH EWES (OR REPLACEMENT RATE) IN NEW ZEALAND ROMNEY MARSH STUD FLOCKS STATIC IN NUMBERS

	1942.	1943.	Total.
Number of stud flocks .. .. .	43	43	86
Number of ewes retained from previous year ..	22,018	22,301	44,319
Number of two-tooth ewes added to the stud flocks	8,661	8,669	17,330
Total number of breeding-ewes .. ..	30,679	30,970	61,649
Average size of stud flocks .. .. .	713.5	720.2	716.8
Range in size of stud flocks .. .. .	..	..	389-1,342
Two-tooths added (or replacement rate), (per cent.)—			
Mean .. .. .	28.23	27.99	28.1
Standard deviation .. .. .	..	..	5.22
Standard error .. .. .	..	..	0.56
Range .. .. .	..	..	17.9-43.7

These data are based on all Romney Marsh stud flocks (ranging in size from 400 to 1,300 ewes) which were static in numbers for three consecutive years, and represent one-fifth of all the breeding-ewes in Romney Marsh studs (*N.Z. Agr. & Pastoral Statistics*, 1943-44). The mean proportion of two-tooth ewes in these stud flocks is 28.1 per cent., which is in very close agreement with the mean proportion of two-tooth ewes in stud A (Tables I and II)—namely, 28.4 per cent. This would indicate that the figures in Table II are probably fairly representative of the age composition of breeding-ewes in New Zealand Romney Marsh studs.

Some other aspects of age will be discussed in subsequent parts of this study.

#### AGE OF BREEDING-RAMS

The age of rams used for breeding seems to be of lesser importance than the age of dams. However, the fact that "the sires are half the flock" should not be overlooked, and information concerning their ages might be of considerable value in assessing the methods adopted for the improvement of stud sheep in this country.

The sources of information were the same as those for the breeding-ewes. The rams in New Zealand Romney Marsh studs which are static in numbers represent about one-seventh of all the registered Romney rams. The average

age composition of rams used in stud A in 1941-44 and those kept in 1942-43 breeding seasons in previously mentioned studs static in numbers are set out in Table IV. In addition, the table contains similar figures for a stud X (1939-43), which is regarded as one of the leading stud flocks in this country.

TABLE IV.—AVERAGE AGE COMPOSITION OF RAMS USED FOR BREEDING IN (i) STUD A (1941-44) AND KEPT IN (ii) STUD X (1939-43) AND IN (iii) NEW ZEALAND ROMNEY MARSH STUDS STATIC IN NUMBERS (1942-43)

Age of Rams.	Stud A (i).		Stud X (ii).		Studs Static in Numbers (iii)	
	Number.	Per Cent.	Number.	Per Cent.	Number.	Per Cent.
Hoggets .. ..	2	2.0	22	20.8	29	2.0
Two-tooth .. ..	37	37.0	51	48.1	404	27.8
Four-tooth .. ..	24	24.0	19	17.9	356	24.5
Six-tooth .. ..	14	14.0	11	10.4	285	19.6
Full-mouth .. ..	10	10.0	3	2.8	182	12.5
5½ years .. ..	9	9.0	..	..	124	8.5
6½ years .. ..	4	4.0	..	..	43	3.0
7½ years and over ..	..	..	..	..	30	2.1
Total .. ..	100	100.0	106	100.0	1,453	100.0

The average ages at mating were : (i) stud A, 35.3 months : (ii) stud X, 22.1 months : and (iii) studs static in numbers, 38.4 months. Some allowance should be made in (ii) and (iii) studs for those rams which were registered in flock-books and not bred from but kept as "reserves." Judging from information set out in Table VI as to the percentage of rams kept in studs, as well as the average number of ewes per ram, it would appear that the proportion of rams kept as reserves is small. It is therefore reasonable to assume that the figures given for those studs (iii) are fairly representative of the average ages of rams used for breeding in Romney Marsh studs in this country. This being so, it is clear that neither A nor, especially, X studs are typical in this respect. The differences, as can be seen from Table IV and Table V, are mostly due to using and culling higher proportions of younger rams.

TABLE V.—AVERAGE "REDUCTION" OF RAMS ACCORDING TO THEIR AGES DUE TO CULLING, MORTALITY, ETC.: (i) 1941-44; (ii) 1939-43; (iii) 1942-43

Age of Rams.	Stud A (i).		Stud X (ii).		Studs Static in Numbers (iii).	
	Number.	Per Cent.	Number.	Per Cent.	Number.	Per Cent.
Two-tooth to four-tooth	37-24=13	35.1	51-19=32	62.7	404-356=48	11.9
Four-tooth to six tooth	24-14=10	41.7	19-11=8	42.1	356-285=71	19.9
Six-tooth to full-mouth	14-10=4	28.6	11-3=8	72.7	285-182=103	36.1
Full-mouth to 5½ years	10-9=1	10.9	..	..	182-124=58	31.9
5½ years to 6½ years ..	9-4=5	55.5	..	..	124-43=81	65.3
6½ years to 7½ years and over	..	..	..	..	43-30=13	30.2

The incidence of registering and presumably using ram hoggets is very small. Out of forty-three breeders of stud flocks static in numbers, eight, or 19 per cent., registered them in the flock-book. The possibility of the progeny-testing of ram hoggets by other stud breeders without registering them could probably be ignored, since very few stud breeders claim to be progeny-testing their rams. Similarly, only ten stud flocks, or 23 per cent., registered seven and a half years and older rams. Indeed, the oldest sires kept were seven rams nine and a half years old, six of which were kept in one stud. Thus it may be said that roughly 80 per cent. of stud breeders use rams which are not younger than one and a half years and not older than six and a half years, or approximately 96 per cent. of all rams kept for breeding fall within this age group.

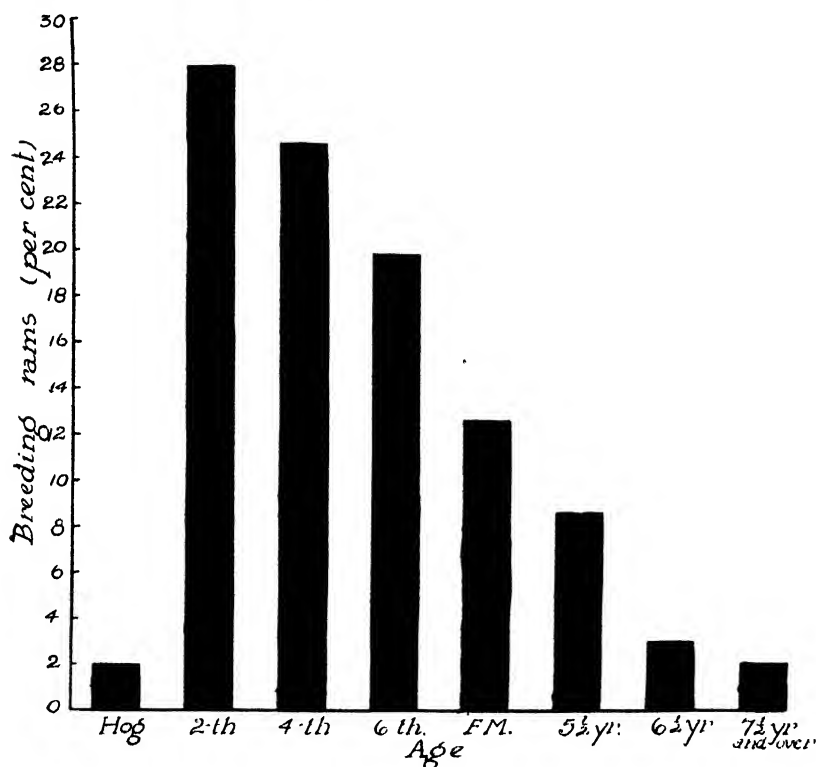


FIG. 2.—Showing percentage age-distribution of breeding-rams.

The average productive lifetime of a stud sire is approximately four years and the average rate of replacement is approximately 30 per cent.

Neglecting, for the sake of comparison, the small proportion of ram hoggets, it is clearly seen that the average age composition of rams (see also Fig. 2) exhibits a trend very similar to that of breeding-ewes, but with a possibly higher concentration of rams up to the age of six-tooth and a more pronounced drop after that age. Similarly, neither the average age (♂ 38.4 months versus ♀ 40 months) nor the average rate of replacement (♂ 30 per cent. versus ♀ 28 per cent.) is very different. These figures lead to a rather unexpected but important conclusion—namely, that the average rate of culling (all other factors being equal) of stud sheep, once they attain

the breeding age and enter the stud flock, is very much the same for both sexes. Against this one might say that, whereas on the average every second ewe hogget is selected each year for replacement, one or two rams are selected from every hundred ram hoggets. Hence the scope for pre-stud selection of rams is comparably greater, resulting in much more careful selection of stud sires before they enter the stud flock. This is undoubtedly true, but as a general rule the selection is based on appearance and pedigree.

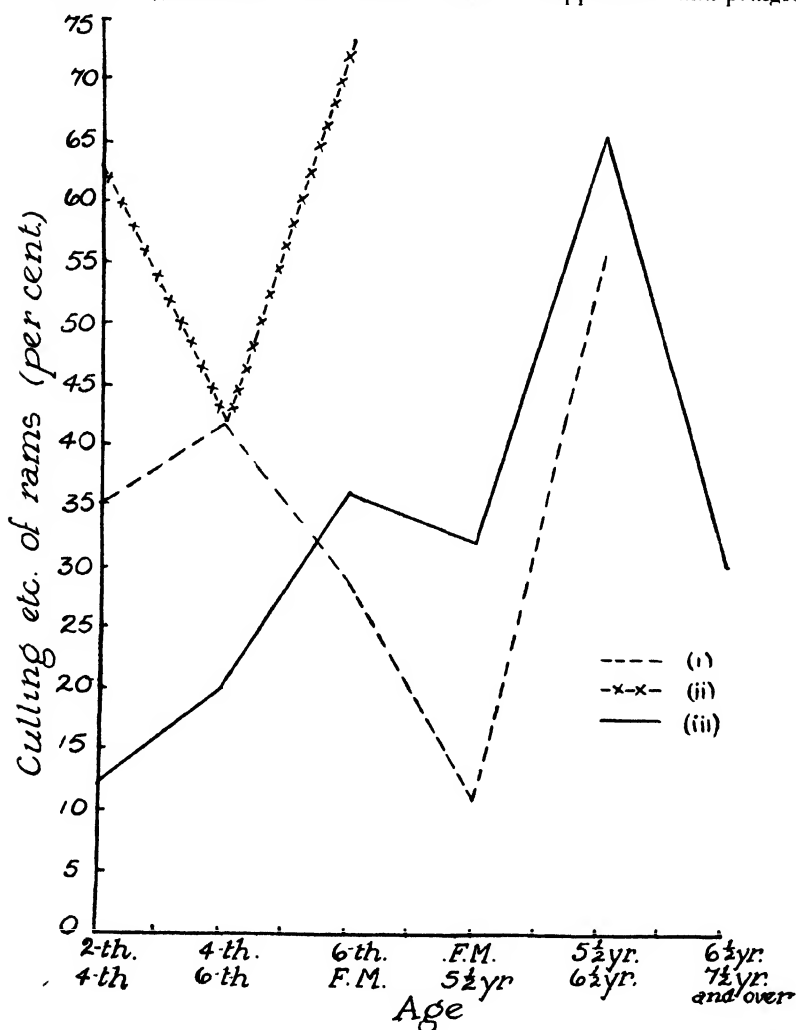


FIG. 3.—Showing average reduction of rams according to their ages due to culling, mortality, &c.

The proportion of sons by progeny-tested sires, let alone progeny-tested sires themselves, entering stud flocks is insignificant.

That the breeding method adopted can influence the proportion of rams falling into different age groups has been illustrated in Table IV. How the age composition of rams can reflect the breeding methods adopted may be seen from Table V and Fig. 3. The table, being based on the previous

one, gives the average proportion of rams which "disappear" between two consecutive breeding seasons. For instance, out of thirty-seven two-tooth rams, only twenty-four reappeared next season as four-tooth, and thirteen disappeared. It is quite safe to assume that they were mostly culled, although some died, met with accidents, &c. It should be noted that in both studs A and X, the breeding policy is based on progeny-testing, and, further, that one-eighth of the sires used in stud X are ram hoggets. This makes any direct comparison between the two studs rather difficult.

For the purpose of the present argument, let us assume that the mortality, &c., was roughly equal in all studs. Thus the differences in the average proportions of rams which have functioned in one season but did not appear in the next would measure the approximate rates of culling according to rams' ages. For instance, out of all two-tooth rams functioning in one season (see Table V), 35.1 per cent. in (i) and 11.9 per cent. in (iii) did not appear in the next season. The difference is 23.2 per cent. In other words, the rate of culling of two-tooth rams is 23.2 per cent. higher in (i) than in (iii).

This illustrates the statement that the method of progeny-testing requires a high proportion of two-tooths and, if possible, ram hoggets to be tested and culled. McMahon (1940) calculated the following figures for the quickest and greatest improvement of progeny in regard to the fleece weight: "culling 80 per cent. rams and 50 per cent. ewes; progeny test 20 per cent. rams, keep 3 per cent." Postulating 100 per cent. lambing and equal sex ratio, it is clear that approximately 94-98 per cent. of rams are discarded prior to entering the stud and only 5-6 per cent. and 2-3 per cent. are progeny-tested in studs X and A respectively. Those of progeny-tested rams which are obvious failures are culled after the first season, while the more promising ones are used once more. Thus, about 62 per cent. of rams in progeny-testing stud A, against 29.5 per cent. of those in studs static in numbers, are not used after the first two seasons. Stud X shows an even higher proportion of rams discarded after the first two seasons, but owing to the use of ram hoggets no direct comparison is possible.

These figures indicate in a general way that-

- (a) Progeny-testing as a method of improvement is far from being prevalent. Indeed, the general trend of age composition for New Zealand Romney studs static in numbers would indicate that once a ram is chosen as stud sire it is apt to be used there barring death, sterility, &c., until it is culled for age and possibly fear of inbreeding. There is little room left for culling on economically important scores.
- (b) Stud A, although culling the sires fairly heavily according to their "gets," seems to be rather restricted in numbers of young rams for progeny-testing.
- (c) Stud X seems to be in a position to progeny-test about 5-6 per cent. of young rams and to cull a higher proportion of two-tooth (a substantial proportion of which is presumably appraised on two crops of lambs). Since 3 per cent. of rams only are used as full-mouth, it would appear that the stud breeder must be satisfied that the young rams are better than the stud's best older ones.
- (d) In so far as the breed is concerned, and speaking from the point of view of a long-range policy, the foregoing picture seems to present great possibilities of further improvement of New Zealand Romney Marsh sheep, for, if it is true that the surest way of live-stock improvement lies in progeny-testing, the use of it is still in its incipient stage, and the potentialities for further improvements would seem to be still mostly untapped.

Admittedly, the foregoing general outline of the breeding policies adhered to by the New Zealand Romney stud breeders, and especially points (a) and (d), is based (except for stud A) on figures gathered from flock-books for all the static in numbers studs from 400 to 1,300 breeding-ewes strong. The sheep in these studs represent approximately one-fifth and one-seventh of Romney stud ewes and rams respectively. Therefore, strictly speaking, the conclusions refer to those studs only, and, further, it should be understood that the general outline may not hold true for individual studs.

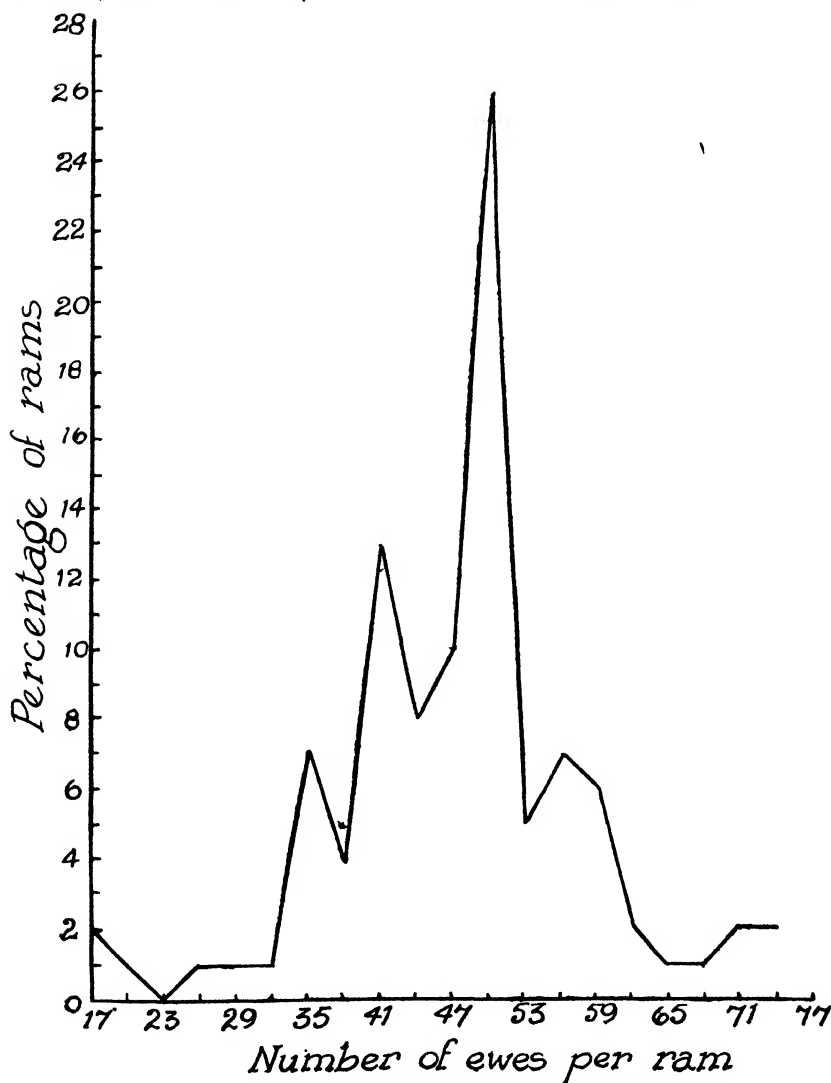


FIG. 4.—Showing average distribution of number of ewes per ram.

#### NUMBER OF EWES PER RAM

For the sake of completeness rather than sequence it is proposed to analyse briefly some figures pertaining to the allotment of ewes per sire in an ordinary paddock mating.

The data set out in Table VI indicate the general utilization of rams for breeding in studs bigger than four hundred sheep and static in numbers. Somewhat fuller information is given for stud A in Tables VII and VIII and also in Figs. 4 and 5.

TABLE VI.—PROPORTION OF RAMS KEPT FOR BREEDING, AND THE AVERAGE NUMBER OF EWES PER RAM IN NEW ZEALAND ROMNEY MARSH STUDS STATIC IN NUMBERS (1942 AND 1943)

Number of breeding-ewes	..	..	..	..	61,649
Number of rams kept	..	..	..	..	1,453
Total number	..	..	..	..	63,102
Rams—					
Percentage of total	..	..	..	..	2.3
Percentage of ewes	..	..	..	..	2.4
Number of ewes per ram—					
Average	..	..	..	..	42.4
Range	..	..	..	..	28.6-116.6

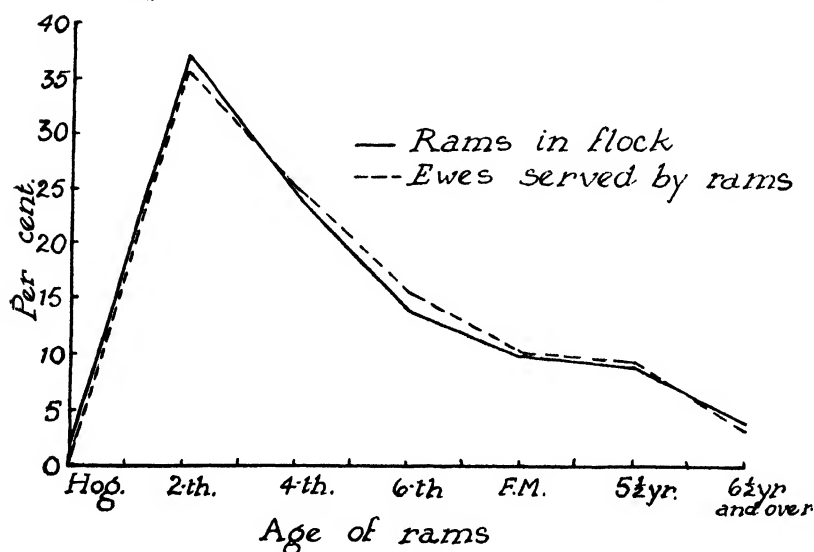


FIG. 5.—Showing percentage of age-distribution of rams and percentage of ewes served by rams of different ages.

TABLE VII.—AVERAGE DISTRIBUTION OF NUMBER OF EWES PER SIRE. (STUD A, 1941-44)

Number of Ewes per Sire.	Number and/or Percentage of Sires.	Number of Ewes per Sire.	Number and/or Percentage of Sires.
From 16 to 18	2	From 49 to 51	26
From 19 to 21	1	From 52 to 54	5
From 22 to 24	0	From 55 to 57	7
From 25 to 27	1	From 58 to 60	6
From 28 to 30	1	From 61 to 63	2
From 31 to 33	1	From 64 to 66	1
From 34 to 36	7	From 67 to 69	1
From 37 to 39	4	From 70 to 72	2
From 40 to 42	13	From 73 to 75	2
From 43 to 45	8		
From 46 to 48	10	Total	100

TABLE VIII.—AVERAGE NUMBER AND PROPORTION OF EWES PUT TO SIRE OF DIFFERENT AGES (STUD A, 1941-44)

Age of Sires.	Sires.		Ewes		Average per Sire.	Range per Sire.
	Number.	Per Cent.	Number.	Per Cent.	Number.	Number.
Hoggets .. .. .	2	2	33	0.70	16.5	16-17
Two-tooth .. . .	37	37	1,091	35.55	45.7	20-65
Four-tooth .. . .	24	24	1,179	24.78	49.1	27-72
Six-tooth .. . . .	14	14	748	15.72	53.4	40-62
Full-mouth .. . .	10	10	485	10.20	48.5	36-75
5½ years .. . . .	9	9	455	9.56	50.6	43-75
6½ years .. . . .	4	4	166	3.49	41.5	30-50
Total .. . . .	100	100	4,757	100.00	47.6	16-75

The mean of the number of ewes per sire in paddock mating is 47.6, while the mode is 50. It is quite possible that the secondary peaks in Fig. 4 indicate the breeder's preference for a certain number of ewes per sire. It will be observed that hoggets, and to lesser extent six and a half years old rams, show a smaller allotment of ewes. The maximum is seventy-five ewes for rams four and a half years to five and a half years old (see Table VII), but the age of the rams, except ram hoggets, is of minor importance in apportionment of ewes. This is clearly illustrated in Fig. 5, where the percentage of total ewes served by sires of different ages is plotted. If each age group of rams had received the same number of ewes, these curves would coincide. As it is, they are almost coincident.

Some other aspects of the reproductive abilities of rams, especially in connection with hand service, will be analysed in subsequent parts of this study.

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## A PROGRESS REPORT ON A COLLECTION OF JAPANESE PERSIMMONS (*DIOSPYROS KAKI* L.f.) AT MOUNT ALBERT, AUCKLAND

By H. M. MOUAT, Pomologist, Plant Diseases Division, Department of Scientific and Industrial Research

[Received for publication, 1st July, 1946]

### Summary

(1) An account is given of a collection of seventeen varieties of Japanese persimmon (*Diospyros kaki* L.f.) planted during the years 1939-40.

(2) Marked differences have been observed in growth, foliage, fruit-drop, and fruit type of different varieties.

A COLLECTION of thirty-two trees of seventeen varieties of Japanese persimmon (*Diospyros kaki* L.f.) obtained from two nurseries in Australia was planted at the Plant Diseases Division, Mount Albert, Auckland, in 1939 and 1940. Varieties included in the collection are shown in Table I. The rootstock of trees from one source was stated to be *D. kaki*. That of the others is unknown, but is probably the same. The soil is a brown crumbly loam of basaltic origin. The trees were spaced 15 ft. apart in a single row, in most cases a pair of each variety being planted together. Approximately every eighth tree was of the Gailey variety, included to ensure good pollination.

TABLE I.—GROWTH AND FLOWERING OF JAPANESE PERSIMMONS

Variety.	Rootstock	Number of Trees.	Year of Planting.	Tree Size	Year of Flowering.
Dai Dai Maru ..	Unknown	1	1939 ..	Medium	..
Flat Sweet ..	"	2	1939 ..	"	1945
Fuyu ..	<i>D. kaki</i> ..	1	1940 ..	"	1945
Gailey ..	" ..	4	1940 ..	Large	1944,* 1945*
Hachiya ..	" ..	2	1939, 1940†	"	..
Hyakume ..	Unknown	1	1939 ..	"	1945
Kurokumo ..	"	2	1939 ..	"	1944, 1945
Miyotan ..	<i>D. kaki</i> ..	2	1939, 1941†	"	1945
Okame ..	" ..	2	1940 ..	"	1944, 1945
Ormond ..	" ..	2	1940 ..	"	..
President Honorarity	Unknown	1	1939 ..	"	1945
Tamopan ..	<i>D. kaki</i> ..	2	1940 ..	Medium	..
Tanenashi ..	" ..	2	1940 ..	"	..
Titibut ..	Unknown	2	1940 ..	Small	..
Tsuru Gaki ..	<i>D. kaki</i> ..	2	1940 ..	"	1944,‡ 1945 ‡
Tsuru Marghari ..	" ..	2	1940 ..	Medium	1944, 1945
Yemon ..	" ..	2	1940 ..	"	1944, 1945

\*Three trees only.

†Re-plant.

‡One tree only.

The trees were slow in becoming established, but are now growing well. Varieties have shown considerable differences in type of growth and tree size (see Table I). Some—e.g., Titibut and Tsuru Gaki—are of dwarf habit, being little over 5 ft. high, while others—e.g., President Honorarity and Okame—are vigorous types now over 8 ft. in height. Ormond is notable for its late season of growth, extending six to eight weeks beyond that of the other varieties.

Differences were also noticed in leaf character, some varieties having very distinctive foliage.

With reference to the flowering habit of Japanese persimmons, Hume (1914) and Hodgson (1942) have shown that varietal differences exist. Whereas nearly all varieties bear pistillate flowers, some do not bear staminate flowers, and of those that do, not all produce them regularly every season. Hume has pointed out that Gailey, which belongs to the group producing both pistillate and staminate flowers regularly, is valuable as a pollinator for the other groups. Although fruit will develop parthenocarpically, seed formation is necessary to prevent excessive drop of immature fruit (Asimi and Chow, 1936). According to Traub and Robinson (1937), Noguchi (1934) found that pollination may also produce a metaxenial effect on fruit shape and the character of non-astringency. Fruit-drop is one of

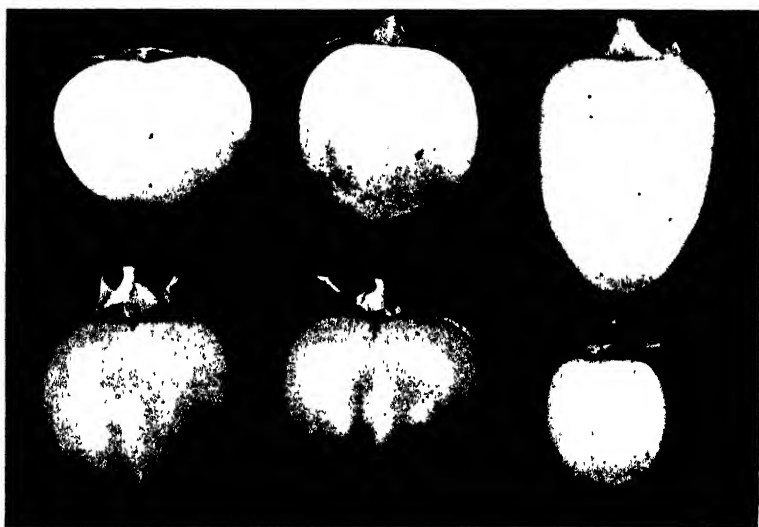


FIG. 1.—Showing variation in size and shape of six varieties of Japanese persimmon. (Top. Yemon, Tsuru Marghari, Tsuru Gaki. Bottom. President Honorarity, Hachiya, Gailey.

[Photo, by R. I. Hughes.

the problems of persimmon growing overseas, where the chief cause is faulty pollination. Hodgson (1942) in California has shown that in the Hachiya variety the drop is accentuated where *Diospyros lotus* L. is used as a rootstock.

Six varieties flowered in 1944 and eleven varieties in 1945 (Table 1). The period of flowering extended over the last two weeks of November and the first week of December. To date the only varieties in the collection that have borne staminate flowers are Gailey and Miyotan. Records over a considerable period will be necessary to determine fully the flowering habit of the different varieties.

All varieties that flowered in 1944 and 1945 set fruit. Drop of immature fruit occurred mainly during the month of February and the first week of March. In the first season the few fruits that set on Okame and Kurokumo all dropped prematurely. The other varieties produced a small crop. In the second season, fruit-drop, which may have been accentuated by the

dry conditions, was very heavy in Kurokumo, Okame, and President Honorarity, and moderately heavy in Tsuru Gaki. There was practically no drop in Gailey, Yemon, and Tsuru Marghari.

The first picking of fruit was made in the first week of May and the last picking in the second week of June. Tsuru Gaki, in spite of the considerable drop of immature fruit, bore a very heavy crop. A good crop was also produced by Tsuru Marghari, moderate crops by Gailey and Yemon, and very light crops by the others.

Wide differences were observed in shape, size, and general characteristics of the fruit from different varieties. Figure 1 shows the variation in size and shape of fruits from six varieties.

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# THE NEW ZEALAND JOURNAL OF SCIENCE AND TECHNOLOGY

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## THE UTILIZATION OF BUTTERMILK IN NEW ZEALAND

By F. H. McDOWALL, Dairy Research Institute (N.Z.), Department of  
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[Received for publication, 18th April, 1946]

### Summary

*Part I.*—In the seasons 1940–44, buttermilk from New Zealand butter-factories was used almost exclusively for animal feeding. From about 90 per cent. of the factories the buttermilk was sold to private pig-farmers. The remaining 10 per cent. utilized the buttermilk on a factory-owned pig-farm. The average returns for the buttermilk over all the factories surveyed was 2s. 10½d. per ton of butter manufactured. The highest average returns were obtained by the smallest and the largest factories. The ten factories owning their own pig-farms obtained, on the average, a return of 5s. per ton of butter manufactured over the four dairying seasons.

*Part II.*—Buttermilk is shown to vary in composition with the composition of the milk from which the cream is derived, the degree of dilution of the cream during processing in the factory, and with the extent of fat loss during churning. On the assumption that the average fat content of the cream at churning in New Zealand factories is 36.0 per cent., that the fat and solids-not-fat contents of the buttermilk are 0.80 per cent. and 7.2 per cent. respectively, and that the buttermilk content of the granules at completion of draining of the buttermilk is 18.0 per cent., 1.304 tons of buttermilk are shown to be discharged per ton of butter manufactured.

From monthly butter-production figures over the years 1943–45 the seasonal variation in quantity of buttermilk discharged daily has been calculated for factories of annual outputs from 500 tons to 6,000 tons of butter.

Separation of a portion of the fat from buttermilk should be possible, but the information available in the literature is not very complete.

Some casein is being manufactured from buttermilk in Australia and possibly also in the United States, but the product is of inferior quality and has limited uses.

The manufacture of semi-solid buttermilk from buttermilk, which is allowed to acidify before evaporation to about 30 per cent. of its original volume, is widely practised in the United States. This product is used for animal food. Condensed sweet-cream buttermilk is used for human food, especially for baking and ice-cream making.

In some countries some buttermilk is used direct for liquid consumption, for use in baking, or is converted to cottage cheese.

Buttermilk may be dried either for human consumption or for animal-feeding purposes. The roller process is commonly used. The cost of drying varies with the conditions of operation. On certain assumptions as to the composition of the buttermilk, the cost of drying at present (March, 1946) in a 2,500-ton factory has been assessed at £21 10s. per ton of powder. The returns for the powder at the factory should therefore be at least £23 10s. per ton to equal the present return for buttermilk utilized on the factory pig-farm.

The annual consumption of whole-milk powder in New Zealand is 800 tons, and of skim-milk powder 1,800 tons. Of the skim-milk powder consumed, 300 tons to 500 tons are used in ice-cream. The total possible production of dried buttermilk in New Zealand from factories of over 1,000 tons annual output of butter is approximately 10,000 tons. The extensive production of buttermilk powder for human consumption within New Zealand would therefore involve the creation of a new market for dried-milk products, or disposal on the export market. The keeping quality under shipping conditions and the method of packaging for export require investigation before the possibility of this channel of disposal can be assessed.

There appears to be a possible market for up to 1,000 tons of dried buttermilk for poultry feeding, and for 800 tons to 1,000 tons for calf feeding, but these markets are not yet developed to their full possibilities. The use of dried buttermilk for pig feeding is scarcely likely to be economic, and the competition of other feeds such as meat meal, available at lower prices, would also restrict the demand for buttermilk powder for this purpose.

## PART I.—SURVEY OF BASIS OF BUTTERMILK DISPOSAL AT NEW ZEALAND FACTORIES

THE problem of the disposal of the buttermilk produced as a by-product in the factory manufacture of butter has two complementary aspects: (1) the buttermilk is a high-quality food product, and should be utilized to the fullest advantage; and (2) some method of utilization is essential, as the discharge of buttermilk into the factory drains would cause serious pollution of the natural streams into which the drainage flows. In New Zealand the buttermilk is used almost exclusively for pig-feeding purposes. The buttermilk is sold either by direct tender for the supply year by year or for a period of years, by tender based on the tonnage of butter, to private pig-farm owners, or is used on a pig-farm owned and operated by the factory company. Hitherto there has been no information available as to the particular method of disposal most generally adopted or as to the terms of disposal.

In 1944, in response to a circular forwarded from this Institute to each butter-factory company, details were supplied of the method of, and of the returns from, buttermilk utilization in the large majority of butter-factories over the four years 1940-44. Only two factories were drying buttermilk. In one of these a portion of the buttermilk was being dried by the roller process and was sold for animal-feeding purposes. In the other factory a portion was being dried by the spray process. Some of the spray-dried product was used for human food, but the greater proportion of this powder also was used for animal feeding. In the tables given below the receipts from buttermilk drying are not included.

## FINANCIAL RETURNS FOR THE DISPOSAL OF BUTTERMILK AT NEW ZEALAND FACTORIES

The results of the survey are given in Table I. The receipts for all methods of disposal have been calculated to the basis of "per ton of butter," and the factories have been classified according to the returns received, in steps of 1s. per ton of butter. It will be seen that, over the four years, the majority of factories (67.9 per cent.) received between 1s. and 3s. per ton of butter for the buttermilk, and only relatively few (18.6 per cent.) received more than 4s. per ton of butter. The maximum return to a factory was 13s. per ton of butter.

TABLE I.—CLASSIFICATION OF FACTORIES ACCORDING TO THE RETURNS FOR DISPOSAL OF BUTTERMILK PER TON OF BUTTER MANUFACTURED, IN STEPS OF 1s. PER TON OF BUTTER

Returns to Factory for Buttermilk per Ton of Butter.				Season, 1940-41.		Season, 1941-42.		Season, 1942-43.		Season, 1943-44.		Seasons, 1940-44.	
				Number of Factories.	Proportion of Total Number of Factories.	Number of Factories.	Proportion of Total Number of Factories.	Number of Factories.	Proportion of Total Number of Factories.	Number of Factories.	Proportion of Total Number of Factories.	Number of Factories.	Proportion of Total Number of Factories.
s.	d.	s.	d.		Per Cent		Per Cent.		Per Cent		Per Cent.		Per Cent
0	0	-	1	14	13.2	12	12.4	11	10.5	10	10.1	47	11.6
1	1	-	2	29	27.4	29	29.9	28	26.9	25	25.2	111	27.3
2	1	-	3	32	30.2	24	24.7	31	29.8	31	31.3	118	29.0
3	1	-	4	8	7.6	18	18.6	13	12.4	16	16.2	55	13.5
4	1	-	5	10	9.4	7	7.3	6	5.8	5	5.1	28	6.9
5	1	-	6	5	4.7	1	1.0	8	7.7	8	8.1	22	5.4
6	1	-	7	5	4.7	3	3.1	1	1.0	1	1.0	10	2.5
7	1	-	8	1	0.9	1	1.0	..	..	2	2.0	4	1.0
8	1	-	9	2	1.9	..	..	1	1.0	1	1.0	4	1.0
9	1	-	10	..	..	1	1.0	2	1.9	..	..	3	0.7
10	1	-	11	..	..	..	..	1	1.0	..	..	2	0.5
11	1	-	12	..	..	1	1.0	1	1.0	..	..	2	0.5
12	1	-	13	..	..	..	..	1	1.0	..	..	1	0.3
Totals ..				106	..	97	..	104	..	99	..	406	..

TABLE II.—AVERAGE RETURNS FOR BUTTERMILK PER TON OF BUTTER CLASSIFIED ACCORDING TO THE TONNAGE OF BUTTER PRODUCED AT THE FACTORY FOR THE YEAR

Yearly Output of Butter.	Number of Factory Seasons.	Average Returns per Ton of Butter.
Tons.		d.
0 - 500	153	55.80
501 - 1,000	70	28.64
1,001 - 1,500	48	31.39
1,501 - 2,000	50	30.24
2,001 - 2,500	25	27.88
2,501 - 3,000	17	39.41
3,001 - 4,000	30	39.63
4,001 -	12	64.92
Average ..	405	34.40

TABLE III.—NUMBER OF FACTORIES OF DIFFERENT YEARLY TONNAGE OUTPUTS (IN STEPS OF 500 TONS) OWNING THEIR OWN PIG-FARMS, AND AVERAGE RETURNS PER TON OF BUTTER FROM THESE FARMS, OVER THE SEASONS 1940-44

Butter Output of Factory.	Number of Factory Seasons.	Average Returns per Ton of Butter
Tons.		s. d.
0 - 500	0	..
501 - 1,000	8	4 8½
1,001 - 1,500	3	6 8
1,501 - 2,000	4	6 3
2,001 - 2,500	6	1 1½
2,501 - 3,000	5	6 7½
3,001 - 4,000	5	4 3
4,001 -	8	6 5
Average ..	39	5 0

### RELATIONSHIP BETWEEN SIZE OF FACTORY AND RETURNS FOR BUTTERMILK PER TON OF BUTTER

The receipts, classified according to the size of factory (yearly output in tons), are shown in Table II. The highest average returns were obtained by the smallest and the largest factories. As will be seen from Table III, there were no factories of under 500 tons with a factory-owned pig-farm. The higher returns for these small factories is no doubt due to the capacity of a nearby farmer to utilize the small quantity of buttermilk in conjunction with the skim-milk from his own farm, without the necessity for concentrating solely on pig-farming. He would thus be able to utilize the buttermilk without being compelled to employ additional labour and to install an elaborate piggery with high installation costs for plant and staff accommodation. The higher returns for the large factories were due to high returns from some of the factories owning and operating their own pig-farm (see Table V). The average return per factory unit per year (not the weighted average) was 34·4d. per ton of butter.

### COMMERCIAL BASIS OF DISPOSAL OF BUTTERMILK

The most common basis of disposal of the buttermilk was at a price per ton or per 100 tons of butter made, but many of the smaller factories sold the whole of the buttermilk for a fixed sum. Some factories, recognizing that the purchaser of the buttermilk would have to undertake a large expense for installation of a pig-farm, sold the buttermilk on a ten-year term. At two factories the buttermilk was given away, in one instance without any *quid pro quo* except that the factory no longer had the responsibility of removing it from the factory premises, and in the other in return for the factory drainage rights through the property of the recipient. During the 1940-41 and the 1941-42 seasons nine, and during the 1942-43 and 1943-44 seasons eleven, of the factories disposed of the buttermilk by means of a factory-owned pig-farm. The average net returns from the operation of the pig-farm are shown in Table III, distributed according to size of the factory.

The returns from the factory-owned pig-farms were thus, on the average, 2s. 1·6d. per ton of butter higher than the average of all returns. For a 2,000-ton factory this would amount to £208 per year, or 2s. 1·6d. per 1,866 lb. of butterfat, equivalent to 1 ton of butter=0·013d. per pound of butterfat. The range of variation in returns from buttermilk per ton of butter for factories of different annual outputs is shown in the scatter diagram in Fig. 1. Returns for factory-owned pig-farms are represented by a cross on the diagram, and study of their position on the diagram again emphasizes the higher returns obtained from the factory-owned pig-farm.

### PIG-FARMING AS A METHOD OF BUTTERMILK UTILIZATION

(a) *Comments on the Results presented above.*—To obtain a balanced appreciation of the figures presented, it is necessary to examine them with the following conditioning factors in mind: (1) prices for pig meats were high during the period under review, whereas many factories sell their buttermilk on a long-term contract, which must allow for the wide fluctuations that do occur in these prices from year to year. (2) The market for pig meats was an assured one during the whole of the period, but this is not

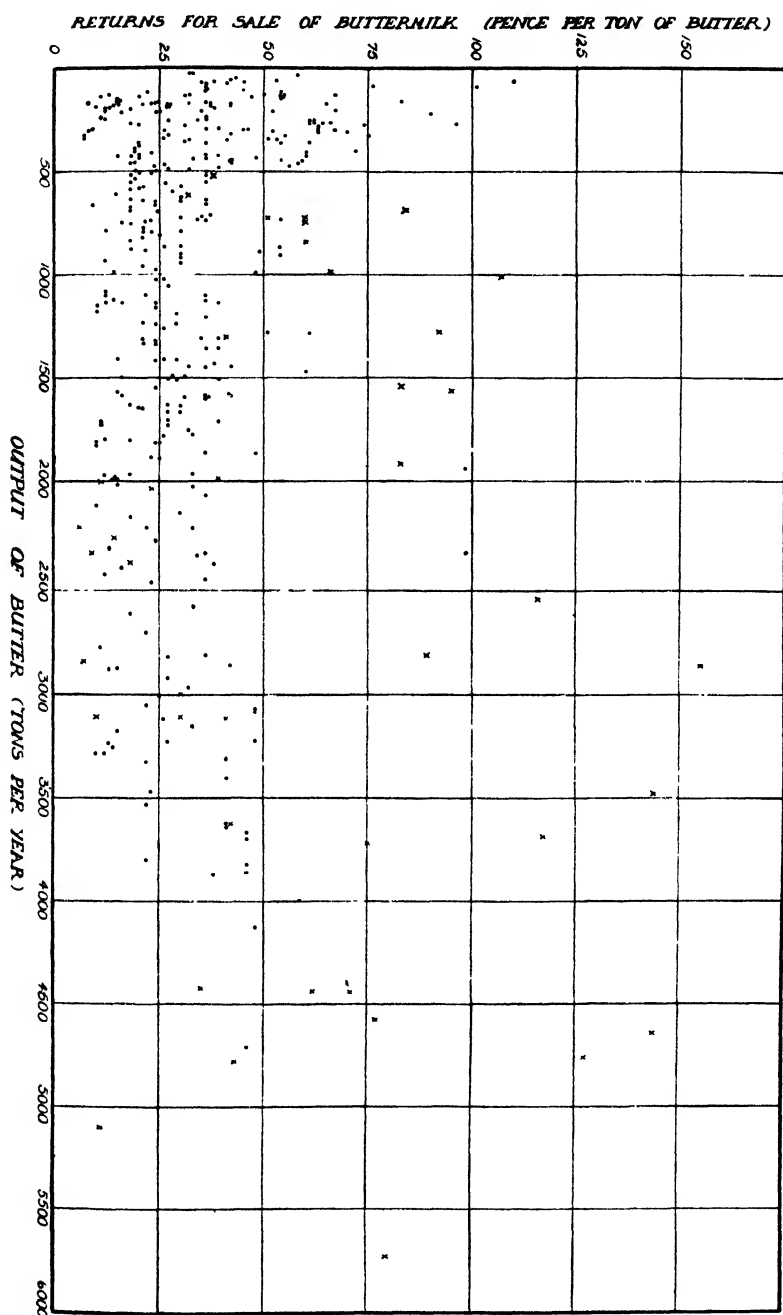


FIG. 1.—Scatter diagram of returns for sale of buttermilk for years 1940-41, 1941-42, 1942-43, and 1943-44, plotted against annual production of butter for the corresponding years.

• = direct sale of buttermilk to private pig-farmer.

x = utilization of buttermilk on factory-owned pig-farm.



always so. (3) Pig-farming on the large scale is more likely to be successful during the first few years of establishment, when the land is fresh. The older the pig-farm, the greater is the care necessary to prevent the area from becoming "pig-sick," with resultant heavy losses of animals from disease. (4) The capital investment for the installation of a properly laid out pig-farm is high. (5) Success in intensive pig-farming is contingent on suitable personnel being available. The manager of the farm must have both a good knowledge of the work, and a real interest in it, if full advantage is to be taken of the fluctuating flow of buttermilk and if losses are to be avoided through the operation of factor (3). Such personnel is not always procurable. (6) No doubt the returns were swollen to some extent from the sale of breeding-pigs to farmers during the period of good demand and good prices for pigs.

*(b) Difficulties in the Utilization of Buttermilk on Pig-farms in New Zealand.*—It will be shown later that the volume of buttermilk discharged from New Zealand butter-factories is subject to marked seasonal fluctuations. Success in full utilization of the supply requires very careful planning of the breeding programme throughout the year. The flush supply of buttermilk occurs in October to December. Litters farrowed in the spring do not reach maturity sufficiently early to take full advantage of the flush supply in October, and it is necessary either to carry store pigs over the winter by means of other feeds so that they shall be ready for the appearance of the buttermilk supply, or to purchase from other breeders. Grain feeds are not easily available in New Zealand at an economic price. Root crops of some kind are necessary to carry the stock over the winter. Some of the larger factory-owned pig-farms have operated on the system of buying in stores in the spring, but this has the serious disadvantages of lack of control of the quality and health of the animals housed on the farm, uncertainty as to the supply and price on the store-pig market, and loss of the profits from the breeding side of pig-farming operations.

The buttermilk as discharged from the factory is very cold, and may cause loss if given to pigs at the temperature of discharge. It has been the experience on some pig-farms utilizing buttermilk that losses of suckling pigs at time of transfer to feeding on buttermilk are higher than on skim-milk farms. The reason has not been clearly shown, but may be related to the temperature of the buttermilk when given to the animals.

*(c) The Efficiency of Utilization of the Food Value of Buttermilk by the Pig.*—Buttermilk is a high-quality food product. The extent of dilution with water is variable according to the factory conditions, but the proportions of the two primary animal foods, protein and carbohydrate, are the same as for skim-milk, which contains 3.5 per cent. to 4 per cent. of protein and 5 per cent. of lactose. For use as an animal food, buttermilk contains a higher proportion of protein than is necessary, and the valuable milk protein is used as a source of energy and for fat production instead of its proper function for flesh and blood production. The most efficient utilization of buttermilk would be obtained by the simultaneous feeding of carbohydrate-rich foods. In New Zealand, however, large supplies of grains are not available at prices which are economic for pig feeding, and where buttermilk is used for pig-farming the inefficient use of its true food value is scarcely avoidable.

## DISCUSSION

Buttermilk from sweet cream is suitable for human food. Its utilization for the feeding of pigs results in the production of only approximately one-tenth of its food value as utilizable human food in the form of pig meat. A large proportion of the food value goes to waste in the form of excreta, and a proportion of that retained is lost as unusable offal when the pig is slaughtered. Apart from these considerations, the data given in Table III show that the financial returns to the butter-factory from the sale of buttermilk are very small. Buttermilk from the churning of sweet cream is an excellent food product, but so far it has not yielded any appreciable economic return to New Zealand butter-factories. In Part II the possibilities of alternative methods of utilization will be examined.

PART II.—THE COMPOSITION OF NEW ZEALAND BUTTERMILK,  
THE VARIATION IN QUANTITY OF BUTTERMILK PRODUCED  
THROUGHOUT THE DAIRYING SEASON, AND A REVIEW OF  
THE POSSIBLE METHODS OF UTILIZATION OF BUTTERMILK

## THE COMPOSITION OF BUTTERMILK

Buttermilk shows quite wide variations in composition, due to variation in the composition of the milk from which it is derived, to variation in the amount of dilution with water during the processing of the cream in the butter-factory, and to variation in the amount of fat lost to the buttermilk in the churning process.

(a) *Composition of Milk*.—Jersey cows yield milk with a high content of solids-not-fat, and the buttermilk from Jersey cream will show a correspondingly high solids-not-fat content. There is also a seasonal variation in solids-not-fat content of milk with change in stage of lactation of the cows from spring to autumn. The values show a rising tendency during the summer and early autumn, but fall in the late autumn on account of the fall in lactose content of milk from cows near the end of lactation. During long spells of dry weather, also, the solids-not-fat content of milk is low. For the purposes of the present review the solids-not-fat content of the serum of the cream from which New Zealand butter is derived (calculated on the fat-free basis) will be taken as 8.8 per cent. (Udy, (1, 2)).

(b) *With Amount of Dilution*.—Some dilution of buttermilk due to addition of water to the cream during processing is unavoidable, but care should be taken in the factory to keep the dilution to a minimum. Excessive dilution means excessive losses of fat in the buttermilk and, in addition, increases the difficulty of economic utilization of the buttermilk. In general, the extent of dilution will depend on (1) the ratio of number of suppliers to factory output (a large number of small suppliers will give increased dilution during the steaming of cream from cans); (2) on the thickness of the cream received (thick acid cream is more difficult to steam out of cream-cans than thin sweet cream, and may require dilution before processing); (3) on the amount of neutralizer required (acid cream requires more neutralizer and therefore greater addition of water as solvent for the neutralizer); (4) on the care taken by the factory staff to avoid dilution (e.g., in washing out neutralizing and holding vats); (5) on the relationship between temperature of ingoing and outgoing cream in the treatment of cream in the vacreator (with preheating before vacreation there is actually a measure of concentration of the cream); and (6) on the amount of break water added

(if any) during the churning process. The average solids-not-fat content found in 1937(3) for buttermilks from five factories using vacreators was 7.35 per cent. Some analyses made more recently gave a rather lower average(4).

(c) *With Variation in Extent of Fat Loss during Churning.*—The fat content of buttermilk is related both to the original fat content of the cream and to the conditions of processing and churning of the cream, and to the season of the year. With increased fat content of the cream there is, in general, an increase in fat content of the buttermilk (but, owing to the smaller volume of buttermilk, the proportion of fat lost in the buttermilk is smaller). Intense vacreator treatment and unduly high temperatures of churning give higher fat losses in the buttermilk, and in the autumn, when the cows are late in lactation and the fat globules are smaller, the fat content of the buttermilk is higher. The average fat content of buttermilk from five commercial factories in 1937(3) was 0.77 per cent., corresponding to a fat content in the unwatered buttermilk of 0.89 per cent. Estimations made in 1945(4) indicate that these results are fairly average for the larger factories where vacreators are in use. In the later discussion it will be assumed that the buttermilk as discharged contains 0.80 per cent. of fat.

#### QUANTITY OF BUTTERMILK FOR DISPOSAL IN NEW ZEALAND FACTORIES AND ITS TOTAL SOLIDS CONTENT PER TON OF BUTTER

For cream of any one fat content at churning, the quantity of buttermilk produced remains fairly constant, but it varies with variation in fat content of the cream. The effect of variation in fat content of the cream from 35 per cent. to 45 per cent. on the quantity of buttermilk produced is shown in Table IV. It has been assumed that butter contains 10 per cent. of buttermilk(Udy (1, 2)), that the fat content of the butter is 81.8 per cent., and that the percentage fat loss from 35 per cent. cream is 1.4, from 40 per cent. cream is 1.2, and from 45 per cent. cream is 0.9.

From 100 parts of cream of 35 per cent. fat content, 98.6 per cent. of the fat is recovered in the butter =  $\frac{98.6}{100} \times 35 = 34.51$  parts.

Butter from 100 parts cream =  $\frac{100}{81.8} \times 34.51 = 42.19$  parts.

Quantity of buttermilk in butter =  $\frac{10}{100} \times 42.19 = 4.22$  parts.

Hence total quantity of buttermilk produced =  $100 - (34.51 + 4.22) = 61.27$  parts.

It is not possible, however, to collect all the buttermilk produced during butter-manufacture. The granules of butter retain a considerable amount of buttermilk which is washed away by the chill-water and is not recoverable on account of the high dilution. The amount of buttermilk retained in the butter will vary with size of granule, churning temperature, softness of butterfat, and, above all, with time allowed for draining. After the treatment with chill-water and careful draining of the granules, the moisture retained in the churn is usually sufficient to give a moisture content of 14 per cent. to 15.5 per cent. in the unfinished butter. It would take up too much time to drain the buttermilk from the granules equally carefully, and the unchilled granules will not drain so easily. It will be assumed in this paper, therefore, that the buttermilk content of the granules at the time of addition of chill-water is equivalent to 18 per cent. of the weight of finished butter. If the finding of Udy (see above) is correct, the loss of buttermilk in the chill-water would then be equivalent to 8 per cent. of the weight of the butter

made. In the above example, then, the quantity of buttermilk discharged, as distinct from the quantity produced, would be—

$$61.27 - \frac{8}{100} \times 42.19 = 57.90.$$

TABLE IV.—QUANTITY OF BUTTERMILK FROM THE CHURNING OF 100 PARTS OF CREAM FOR CREAMS OF DIFFERENT FAT CONTENTS

Fat Content of Cream.	Fat Loss.	Quantity of Butter.*	Total Quantity of Buttermilk.*	Quantity of Buttermilk discharged.*	Ratio of Quantity of Buttermilk discharged to Quantity of Butter.	Fat Content of Buttermilk.
Per Cent.	Per Cent.					Per Cent.
35	1.4	42.19	61.27	57.90	1.37	0.80
40	1.3	48.26	55.70	51.84	1.07	0.93
45	1.1	54.42	49.95	45.60	0.84	1.01

\*Figures represent parts per 100 parts of original cream.

The figures in Table IV show that cream of 35 per cent. fat content at churning yields 1.63 times as much free buttermilk as cream of 45 per cent. fat content. There is then no figure directly applicable for calculation of volume of buttermilk from the figure for quantity of butter made, unless the fat content of the cream at churning is known. The fat content of the cream as received at the factories in New Zealand is fairly uniform at about 39 per cent. If it be assumed that there is no watering of this cream on the farm, the total solids content of the buttermilk produced can be roughly estimated on the assumption that the unwatered buttermilk contained 8.8 per cent. of total solids. A certain amount of dilution in processing is unavoidable, as already pointed out, but some control of the extent of dilution is possible in the factory operation. For the purposes of this paper the fat content of the cream at churning will be taken as 36 per cent. This is equivalent to an increase in volume of 100 parts of cream to  $\frac{39}{36} \times 100 = 108.3$  parts, or an 8.3 per cent. dilution (the 36 per cent. cream

contains  $\frac{8.3}{108.3} \times 100 = 7.7$  per cent of added water). After the churning process, this dilution is transferred almost completely to the buttermilk.

100 parts of original cream of 39 per cent. fat content contained 61 parts of unwatered buttermilk.

108.3 parts of diluted cream containing 39 parts fat contain 69.3 parts of watered buttermilk.

The buttermilk has therefore been diluted by 8.3 parts in 61, or by 13.6 per cent. The original solids-not-fat content of the buttermilk—viz., 8.8 per cent.—now becomes 7.7 per cent. The yield of butter from 100 parts of cream of 36 per cent. fat content, on an assumed fat loss of 1.3 per cent. of the fat in the cream, would be 43.4 parts (calculated as outlined above) and the volume of buttermilk discharged would be 56.6 parts. Hence weight of buttermilk per ton of butter would be 1.304 tons. On the assumption that the fat content of the buttermilk were 0.80, the total solids content of the buttermilk discharged from the manufacture of 1 ton of butter would be  $1.304 \times \frac{8.5}{100} = 0.111$  tons and the water content 1.193 tons. Recent

analyses of buttermilks from a number of factories indicate that the content of solids-not-fat is not as high as 7.7 per cent. when the butter is churned from cream containing 36 per cent. fat. This would mean that there has been a measure of dilution of the cream with water on the farm, due probably to entrance of water into the cream during the flushing of the milking-machine and the separator bowl. The economics of the drying of buttermilk is closely related to the degree of dilution of the solids present, and it is of importance that the assumed average value for solids content shall not be unduly high. For the purposes of the later discussion, therefore, the solids-not-fat content of the buttermilk will be taken as 7.2 per cent., making, with a fat content of 0.8 per cent., a total solids content of 8.0 per cent. The total solids content of the buttermilk from the manufacture of 1 ton of butter would then be 0.104 tons and the water content 1.2 tons.

The assumptions on which the subsequent discussion is based may now be summarized as follows:—

- (1) The fat content of cream at churning is 36 per cent.
- (2) The fat content of the buttermilk is 0.80 per cent.
- (3) The solids-not-fat content of the buttermilk is 7.2 per cent.
- (4) The buttermilk content of the granules at completion of draining of buttermilk is 18 per cent.
- (5) Deduced from the assumptions (1) to (4), the production of 1 ton of butter results in the discharge of 1.304 tons of buttermilk, of which 0.104 ton is buttermilk solids and 1.2 tons are water; or the churning of 1 ton of butter yields 292 gallons of buttermilk, which contain 269 gallons of water.

These assumptions represent an average, but they will not apply consistently in any factory from day to day. The error will vary with the seasonal variations in solids-not-fat content of milk, with the condition of cream treatment in the factory, and with variations in a number of other farm and factory practices. For factories using flash-pasteurizers or some form of preheater before vacreation, the values may be too low. For factories not using preheaters before vacreation, the values may be too high. Any factory contemplating the drying of buttermilk should base the estimate of costs on the analysis of buttermilk as discharged under the current system of cream treatment.

#### SEASONAL VARIATION IN THE QUANTITY OF BUTTERMILK AVAILABLE IN NEW ZEALAND FACTORIES

The seasonal nature of dairying for butter-manufacture in New Zealand introduces a serious obstacle to the efficient economic utilization of buttermilk. Even when the buttermilk is used for animal-feeding purposes the difficulty applies (see Part I), as there are no easily available cheap supplementary grain feeds to replace the buttermilk during the off season. In the drying of buttermilk the seasonal nature of the supply imposes an unduly high daily capacity in a plant erected to cope with the whole of the buttermilk supply from a factory during the flush months.

The quantity of buttermilk produced can be calculated approximately, as shown above, from the quantity of butter produced. In Table V the monthly butter-production figures for New Zealand, divided into four districts are given\* for the years 1943–45. Since most of the South Island butter-factories are small and the seasonal change in these districts is different from that of the North Island districts, only the figures for the three North Island districts are used in the totals and in the subsequent discussion.

\*Figures for individual factories kindly made available by Internal Marketing Division.

TABLE V.—PRODUCTION OF BUTTER EACH MONTH DURING THE DAIRYING SEASONS, 1942-45, IN THE AUCKLAND, TARANAKI, AND WELLINGTON DISTRICTS

Month.	1943-44.				1944-45				1943-45.			1943-44.	1944-45.
	Auckland.	Taranaki.	Wellington.	Totals.	Auckland.	Taranaki.	Wellington.	Totals.	Average for Three Districts				
									Monthly	Daily			
June ..	Tons. 827.8	Tons. 214.2	Tons. 203.4	Tons. 1,245.4	Tons. 1,325.9	Tons. 230.6	Tons. 321.6	Tons. 1,878.1	Tons. 1,561.7	Tons. 52.1	Tons. 285.0	Tons. 233.6	
July ..	1,549.7	229.9	207.1	1,986.7	1,694.1	227.9	248.3	2,170.3	2,078.5	67.0	113.0	120.2	
August ..	5,091.6	438.5	743.8	6,273.9	5,978.2	418.6	818.9	7,215.7	6,744.8	217.6	162.2	193.1	
September	8,661.3	705.1	1,386.7	10,753.1	10,020.4	800.5	1,733.0	12,553.9	11,653.5	388.5	481.4	599.5	
October ..	12,200.1	1,036.6	2,081.0	15,317.7	13,633.1	1,194.4	2,495.3	17,322.8	16,320.3	526.5	962.7	1,221.7	
November	14,143.9	1,205.9	2,523.6	17,873.4	14,932.6	1,325.3	2,745.5	19,003.4	18,437.9	614.6	1,532.4	1,624.7	
December	14,286.8	1,207.5	2,525.7	18,020.0	14,849.2	1,352.0	2,735.6	18,916.8	18,468.4	595.8	1,601.7	1,688.2	
January ..	11,686.7	977.5	1,961.0	14,625.2	14,106.7	1,261.0	2,479.3	17,847.0	16,236.1	523.7	1,342.4	1,636.2	
February	9,038.9	785.2	1,539.8	11,363.9	11,531.7	1,030.0	1,980.9	14,542.6	12,953.3	454.5	1,103.3	1,284.7	
March ..	9,424.5	804.5	1,388.1	11,617.1	10,877.9	984.5	1,930.5	13,792.9	12,705.0	409.8	1,152.1	1,224.2	
April ..	7,656.8	654.9	1,203.3	9,515.0	7,866.0	758.7	1,465.1	10,089.8	9,802.4	326.7	859.8	897.3	
May ..	4,099.1	536.6	850.6	5,486.3	3,901.5	511.8	785.6	5,198.9	5,342.6	172.3	646.0	577.6	
Totals	98,667.2	8,796.4	16,614.1	124,077.7	110,717.3	10,075.3	19,739.6	140,532.2	132,304.5	4,349.1	10,241.9	10,301.0	

In Table VI these figures are reduced to the form of percentages of the respective total annual productions. The figures in Table VI show that the seasonal change in butter-production has the same general trend in all three districts. The higher proportion of butter-production in June and July in Taranaki as compared with the other districts, is no doubt due to the transfer of supply from cheese-factories to butter-factories, which would be greater in proportion in Taranaki than in the other two districts during the winter months.

From the point of view of utilization of buttermilk, the amount discharged daily is of greater significance than the monthly discharge. In the second last column of Table VI, the proportionate discharge per month has been converted to proportionate daily discharge (allowing for the difference in number of days per month, and for 29 days in February, 1944), and in the last column the average daily production for the month, calculated as a percentage of the average daily production for the peak month, is given.

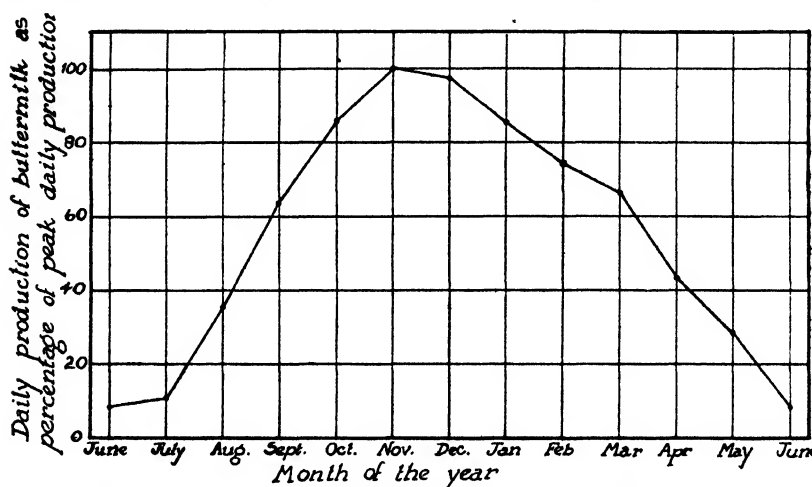


FIG. 2.—Daily production of buttermilk (average for month) in New Zealand factories as percentage of peak daily production (average for month) for each month of the year.

These figures have been plotted in Fig. 2. The correction for the effect of variation in number of days per month removes the major portion of the break in the line joining gross monthly productions, but there is still a slight variation from a smooth line in the transition from February to March, probably due to the climatic conditions in March, 1943, causing an actual rise in daily production figures for March as compared with February.

From the figures in the second last column of Table VI, the monthly productions of butter in a 2,000-ton butter-factory have been calculated (see Table VII). These are converted to daily productions in the next column of the table. Hence on the assumptions previously made that 1.304 tons of buttermilk are discharged for every ton of butter made, the daily production of watered buttermilk has been calculated, and, on the assumption that this buttermilk contains 8.0 per cent. of total solids, the amount of total solids and water in the buttermilk discharged daily have been deduced.

From the data in Table VII, the volumes of buttermilk produced daily for each month of the year have been calculated for factories ranging in output from 500 tons to 6,000 tons (see Table VIII) and these figures have been plotted in Fig. 3. The tables and figure will be referred to later, in the discussion on the drying of buttermilk.

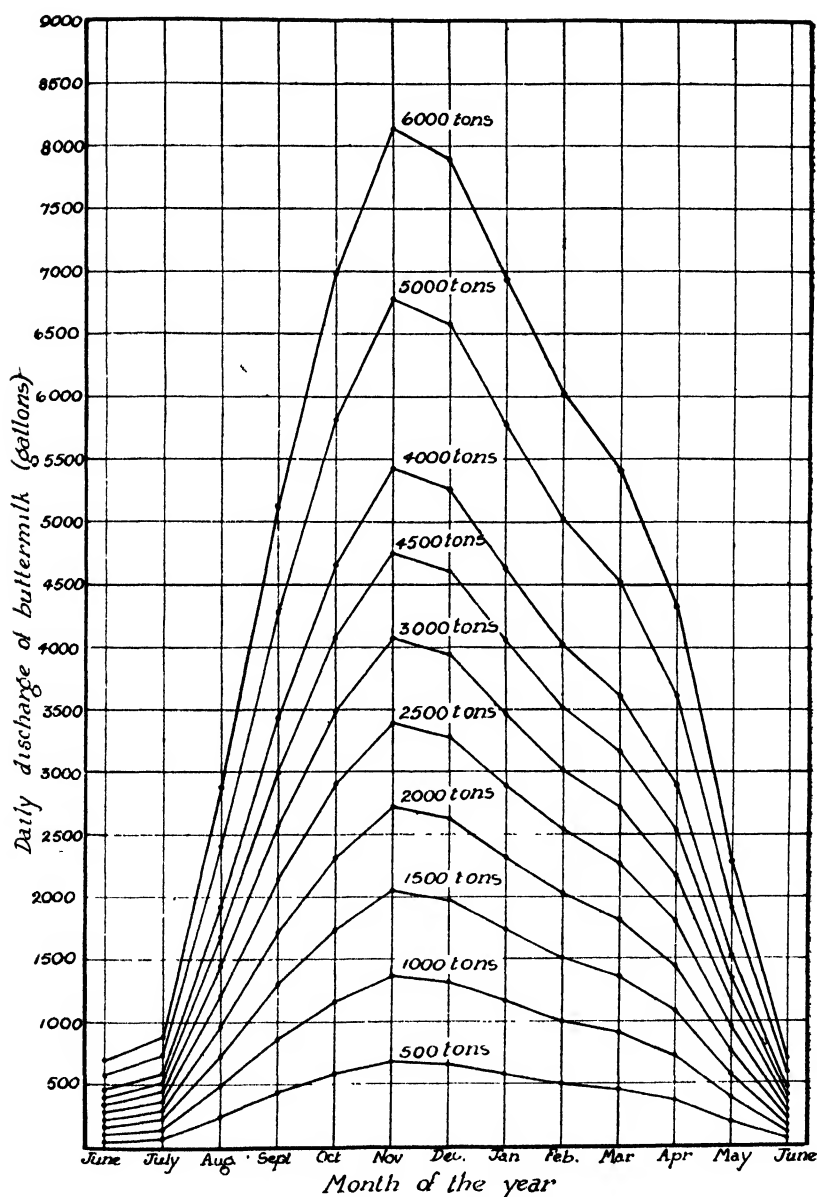


FIG. 3.—Lines showing daily discharge of buttermilk at assumed rate per ton of butter for each month of the year for factories of different yearly outputs for the years 1943–1945 for the North Island districts.



TABLE VI.—MONTHLY BUTTER-PRODUCTION FOR THE AUCKLAND, TARANAKI, AND WELLINGTON DISTRICTS, AS A PERCENTAGE OF THE TOTAL YEARLY PRODUCTION FOR THE DISTRICTS

Month.	1943-44.				1944-45.				1943-45.	Daily Production as a Percentage of Peak Monthly Average Daily Production.	
	Auckland.	Taranaki.	Wellington.	All Districts.	Auckland.	Taranaki.	Wellington.	All Districts.	All Districts, Proportionate Daily Production each Month.		
June ..	0.84	2.44	1.22	1.00	1.20	2.28	1.63	1.33	1.18	1.2	8.5
July ..	1.57	2.61	1.25	1.60	1.53	2.26	1.26	1.54	1.57	1.5	10.6
August ..	5.16	4.98	4.48	5.06	5.40	4.15	4.15	5.13	5.10	5.0	35.5
September ..	8.78	8.02	8.35	8.67	9.05	7.95	8.78	8.93	8.81	8.9	63.3
October ..	12.36	11.78	12.53	12.35	12.31	11.85	12.64	12.33	12.34	12.1	85.8
November ..	14.33	13.71	15.19	14.41	13.49	13.15	13.91	13.52	13.94	14.1	100.0
December ..	14.48	13.73	15.20	14.52	13.41	13.22	13.86	13.46	13.96	13.7	97.2
January ..	11.84	11.11	11.80	11.79	12.74	12.52	12.56	12.70	12.27	12.1	85.8
February ..	9.16	8.93	9.27	9.16	10.42	10.22	10.04	10.35	9.79	10.5	74.5
March ..	9.55	9.15	8.35	9.36	9.82	9.77	9.78	9.81	9.60	9.4	66.7
April ..	7.76	7.45	7.24	7.67	7.10	7.53	7.42	7.18	7.41	7.5	33.1
May ..	4.15	6.10	5.12	4.42	3.52	5.08	3.98	3.70	4.04	4.0	28.3

TABLE VII.—QUANTITY, TOTAL SOLIDS, AND WATER CONTENT OF BUTTERMILK PRODUCED DAILY IN A 2,000-TON BUTTER-FACTORY

Month.	Monthly Percentage Yearly Production	Production of Butter in 2,000-ton Butter-factory		Daily Discharge of Buttermilk		Buttermilk Solids present in Daily Buttermilk Discharge.		Water present in Daily Buttermilk Discharge.	
		Monthly.	Daily.	Tons.	Gallons.	Tons.	lb.	Tons.	Gallons.
June ..	1.18	23.6	0.79	1.03	231	0.082	184	0.94	211
July ..	1.57	31.4	1.01	1.32	296	0.105	235	1.21	271
August ..	5.10	102.0	3.29	4.29	961	0.342	766	3.95	885
September ..	8.81	176.2	5.87	7.65	1,714	0.610	1,366	7.04	1,577
October ..	12.34	246.8	7.96	10.38	2,325	0.828	1,855	9.55	2,139
November ..	13.94	278.8	9.29	12.11	2,713	0.966	2,164	11.15	2,498
December ..	13.96	279.2	9.01	11.75	2,632	0.946	2,119	10.81	2,421
January ..	12.27	245.4	7.92	10.33	2,314	0.824	1,846	9.50	2,128
February ..	9.79	195.5	6.87	8.96	2,007	0.714	1,599	8.24	1,846
March ..	9.60	192.0	6.19	8.07	1,808	0.644	1,443	7.43	1,664
April ..	7.41	148.2	4.94	6.44	1,443	0.514	1,151	5.93	1,328
May ..	4.04	80.8	2.61	3.40	762	0.271	607	3.13	701

TABLE VIII.—VOLUME OF BUTTERMILK PRODUCED DAILY AT BUTTER-FACORIES OF DIFFERENT ANNUAL OUTPUTS OF BUTTER DURING EACH MONTH OF THE YEAR

Month.	Annual Output of Factory (Tons of Butter).									
	500.	1,000.	1,500.	2,000.	2,500.	3,000.	3,500.	4,000.	5,000.	6,000.
June	Gallons. 58	Gallons. 116	Gallons. 174	Gallons. 231	Gallons. 289	Gallons. 347	Gallons. 405	Gallons. 463	Gallons. 579	Gallons. 695
July	74	148	222	296	370	444	518	592	740	888
August	240	481	721	961	1,201	1,442	1,682	1,922	2,403	2,884
September	429	857	1,286	1,714	2,143	2,571	3,000	3,428	4,285	5,142
October	581	1,163	1,744	2,325	2,906	3,488	4,069	4,650	5,813	6,976
November	678	1,357	2,035	2,713	3,391	4,070	4,748	5,426	6,783	8,140
December	658	1,316	1,974	2,632	3,290	3,948	4,606	5,264	6,580	7,986
January	579	1,157	1,736	2,314	2,893	3,471	4,050	4,628	5,785	6,942
February	502	1,004	1,505	2,007	2,509	3,010	3,512	4,014	5,018	6,022
March	452	904	1,356	1,808	2,260	2,712	3,164	3,616	4,520	5,420
April	361	722	1,082	1,443	1,804	2,165	2,525	2,886	3,608	4,330
May	191	381	572	762	953	1,143	1,333	1,524	1,905	2,276

## DISCUSSION OF POSSIBLE METHODS OF UTILIZATION OF BUTTERMILK

## SEPARATION OF FAT FROM BUTTERMILK

The loss of butterfat in the buttermilk in New Zealand factories varies from 0.7 per cent. to 2 per cent. of the fat received. This is a very high rate of loss and it is worth while to consider whether any portion of the fat is recoverable. In cheese-factories receiving as little as one vat of milk daily (800–1,000 gallons) the whey is separated, although it contains only about 0.3 per cent. of butterfat. The fat content of whey can be reduced on separation to 0.03 per cent. It will be seen from Table VIII and Fig. 3 that a 2,000-ton butter-factory discharges more than 700 gallons of buttermilk for nine months in the year. If the larger quantity of fat in the buttermilk as compared with whey were recoverable, the separation should be worth while economically. Separation of buttermilk, however, is more difficult than separation of whey, for several reasons.

(a) *The Nature of the Butterfat in Buttermilk.*—The fatty material in buttermilk contains a much higher proportion of phospholipid compounds than the fat of butter, due to the liberation, during the churning process, of phospholipids adsorbed on the fat globules. The higher the fat content of the cream at churning, the higher the content of phospholipids in the buttermilk. Holm, Wright, and Deysher(5) give the distribution of phospholipid between cream and buttermilk as is shown in Table IX, and

TABLE IX.—DISTRIBUTION OF PHOSPHOLIPID BETWEEN CREAM AND BUTTERMILK(5)

Cream.		Buttermilk Phospholipid Content.
Fat Content.	Phospholipid Content.	
Per Cent.	Per Cent.	Per Cent.
27.0	0.224	0.284
34.0	0.269	0.318
42.0	0.284	0.382

Bird(6) states that on the average 21 per cent. of the fat in buttermilk is phospholipid. The phospholipids are fairly water soluble and would not be separable by centrifuging. Even if all true butterfat were recoverable, therefore, a considerable residue of fatty materials in the buttermilk could be expected.

(b) *Size of the Fat Globules in Buttermilk.*—Sirks(7) has shown that buttermilk contains some large fat globules, but that much of the fat is present in globules too small to be visible under the microscope. In addition, the pasteurizing and churning processes appear to destroy the capacity of the globules to form clusters, as evidenced by the absence of appreciable gravity separation of fat from buttermilk.

(c) *Deposition of Sludge.*—Buttermilk when separated gives greater deposition of sludge in the bowl than whole milk, especially if the cream before pasteurization had developed acidity.

## REVIEW OF WORK OVERSEAS ON THE SEPARATION OF FAT FROM BUTTERMILK

In 1890, Meyers(8), at the West Virginia Agricultural Experiment Station, concluded that butterfat could be recovered from buttermilk when the losses were excessive, but when butterfat losses were low the separation of buttermilk was no longer advisable. Coulter and Combs(9) report trials

by the Division of Dairy Husbandry with hand and power separators and with a high speed super-centrifuge. The results, summarized in Table X, show that the fat content of the separated buttermilk could not be reduced

TABLE X.—RESULTS OF TRIALS ON SEPARATION OF FAT FROM BUTTERMILK (AFTER COULTER AND COMBS(9))

Trial.	Weight of Buttermilk.	Fat Content of Buttermilk.	Weight of Fat recovered as Cream.	Fat Content of Skimmed Buttermilk.
	lb.	Per Cent.	lb.	Per Cent.
1	1,400	0.46	0.665	0.38
2	1,756	0.42	0.937	0.36
3	1,394	0.46	1.23	0.376
4	1,311	0.48	0.96	0.38
5	1,761	1.28	11.76	0.52
6	1,897	1.40	12.76	0.43

below 0.35 per cent., and that the higher the butterfat content of the buttermilk, the higher was the fat content of the separated buttermilk. From the results of Holm, Wright, and Deysher quoted above, it would be expected that the buttermilk from high fat-content creams would, after separation, have a higher fat content than the buttermilk from low fat-content creams. The general conclusion of the American workers was that separation of buttermilk is not practicable unless sweet-cream buttermilk is available in large quantities (not less than 2,500 gallons daily) or in cases where the butterfat content of the buttermilk is relatively high. They report that the process was proving a success in a buttermilk-drying plant where the buttermilk from sixteen to twenty creameries was collected and passed through a large factory separator before drying.

Hunziker(10) reports that efficient separation of buttermilk requires more intense exposure to centrifugal force, such as may be provided by super-speed separators or by providing means to prolong exposure of the buttermilk to centrifugal force. Rushton(11) increased the time of retention in the separator bowl by reducing the orifices of the cream and skim-milk outlets. When the time of retention was increased twenty-fold, Rushton(11) claimed to be able to recover 55 per cent. of the total fat in the buttermilk in the form of a liquid testing 2 per cent. of fat or more.

Mohr, Arbes, and Kelting(12), using a closed foamless Westphalia centrifuge of 200 gallons per hour capacity, obtained from sour-cream buttermilk containing 0.30 per cent. to 0.60 per cent. of fat a buttermilk containing 0.12 per cent. to 0.20 per cent. of fat. In order to avoid curdling the buttermilk, the separation was carried out at low temperatures (57° F. to 61° F.). They state that the buttermilk creams should not contain more than 18 per cent. to 20 per cent. of fat, and the valves of the centrifuge should be adjusted to obtain this condition. Buttermilk with fat content above 1.4 per cent. yielded separated milk containing 0.4 per cent. to 0.45 per cent. of fat. Mohr and Kelting(13) state that the buttermilk cream can be used directly as cream or can be used in buttermaking, either alone or mixed with other cream. Buttermilk cream did not churn as quickly as ordinary cream and the granules retained the moisture more tenaciously. For successful churning, yielding a buttermilk with fat content of 0.5 per cent., it was necessary to cool buttermilk cream containing 18 per cent. fat for twenty hours at 39° F., and to churn at 43° F. Water content below 18.0 per cent. in the butter was obtained only when the butter was cooled at 41° F. for twenty-four hours after salting and preliminary working, and was then

finally worked at 43° F. to 46° F. The butter was softer than normal butter, and foamed and turned brown more easily when heated, owing to the higher lecithin content. Buttermilk cream mixed with an equal quantity of ordinary cream churned satisfactorily, but the tendency to moisture retention was greater than for normal cream. The butters from all-buttermilk cream and from buttermilk and ordinary cream mixed in equal parts were weaker in body than normal cream butters and were of doubtful keeping quality. The findings of Mohr and his co-workers refer to acid-cream buttermilk and are not necessarily applicable to New Zealand sweet-cream buttermilk.

Marek and Brtnik(14) have studied the separation of buttermilk in detail, but the results of their studies are not available in New Zealand.

Capstick(15) reports that buttermilk is separated in some factories in England.

Alfa-Laval Separator Co.(16) have patented a separator bowl for the separation of buttermilk from acid cream. The bowl has holes in the outside casing permitting a portion of the sludge to escape, and so reducing the amount of sludging-up of the separator bowl. The same result would probably be achieved with one of the modern self-desludging bowls. There is no record in the literature of the use of one of these bowls in commercial practice for the separation of buttermilk.

From the above review of the literature it is clear that the separation of fat from sweet-cream buttermilk is possible, but that it is not likely to become economic unless the fat content of the buttermilk is high. The buttermilks from the high-fat creams as churned in New Zealand are not likely to be separable to a fat content lower than 0.4 per cent. In Table XI the quantity of fatty materials in the day's discharge of buttermilk of different fat contents from a 2,000-ton butter-factory are listed. In the assumed standard buttermilk (see above: 0.8 per cent. of fat) the largest daily loss of fat to the buttermilk would be 217 lb. during November, and if 0.4 per cent. of the 0.8 per cent. fat be regarded as unrecoverable, the maximum potential recovery of fat in any one day would be 108 lb., equivalent to 130 lb. of butter. If separation of buttermilk were introduced in New Zealand, the buttermilk cream could possibly be best disposed of by inclusion with whey cream, but even disposal in this way would be contingent on proof, by keeping-quality trials, that the keeping quality of the whey butter was not diminished. The maximum value of the day's recovery, without allowing for costs of separation or costs of reconversion to butter, would be  $130 \times 1/2 = £7$  11s. 8d. at the guaranteed price for whey butter. The total amount of recoverable butterfat, on the assumption that separation was carried out only during the months August to May would be 22,727 lb., equivalent to 27,272 lb. or 244 cwt. of butter with a value of approximately £1,635 at the basic price of 14.39d. per pound of whey butter.

The price of a separator suitable for the separation would be, at 1946 prices, £750, and this machine, running at half normal capacity to give improved separation, would separate the 606,070 gallons of buttermilk in 1,100 hours of running. On these assumptions, the cost of separation may be estimated as follows:—

Interest at 5 per cent., and depreciation and maintenance at 10 per cent. on separator, pumps and fitting, cream-cans, &c., at, say, £1,000 .. .. .	£	s.	d.
Power costs—			
Separator, 1,110 hours at 3 h.p.			
Pump, 1,100 hours at 1½ h.p.			
5,000 kWh. at 1d. per unit .. .. .	21	0	0
Labour, say 1 man at 4 hours per day = 310 × 12s. ..	186	0	0
	<hr/>		
	£357	0	0

TABLE XI.—QUANTITY OF BUTTERFAT IN BUTTERMILKS DISCHARGED DAILY FROM A 2,000-TON BUTTER-FACTORY FOR DIFFERENT PERCENTAGE FAT CONTENTS OF THE BUTTERMILK

Month.	Daily Butter-production.	Daily Discharge of Buttermilk.	Quantity of Butterfat in Buttermilk discharged Daily when the Percentage Fat Content of the Buttermilk is—						
			0.2.	0.4.	0.6.	0.8.	1.0.	1.2.	1.4.
June ..	0.78	Gallons.	lb.	lb.	lb.	lb.	lb.	b.	lb.
July ..	1.01	231	4.6	9.2	13.9	18.5	23.1	27.7	32.3
August ..	3.29	296	5.9	11.8	17.8	23.7	29.6	35.5	41.4
September ..	5.87	961	19.2	38.4	57.7	76.9	96.1	115.3	134.5
October ..	7.96	1,714	34.3	68.6	102.8	137.1	171.4	205.7	240.0
November ..	9.29	2,325	46.5	93.0	139.5	186.0	232.5	279.0	325.5
December ..	9.01	2,713	54.3	108.5	162.8	217.0	271.3	325.6	379.8
January ..	7.92	2,314	46.3	92.6	138.8	185.1	231.4	277.7	324.0
February ..	6.87	2,007	40.1	80.3	120.4	160.6	200.7	240.8	281.0
March ..	6.19	1,808	36.2	72.3	108.5	144.6	180.8	217.0	253.1
April ..	4.94	1,443	28.9	57.7	86.6	115.4	144.3	173.2	202.0
May ..	2.60	762	15.2	30.5	45.7	61.0	76.2	91.4	105.7

The margin left to cover cost of butter-manufacture from the cream would thus be £1,278. If, therefore, the separation could be satisfactorily achieved to the extent of recovery of 50 per cent. of the fat in the buttermilk, the process should be an economic one.

Separation of the buttermilk would, however, introduce a certain amount of inconvenience into the running of the factory, and may involve the construction of additional buttermilk-storage space. Few butter-factories have buttermilk sumps of sufficient capacity to hold more than a small proportion of the buttermilk produced at the height of the season. The buttermilk as it flows from the churn is pumped direct to an adjacent pig-farm or to a large outside storage tank from which it is collected by the contracting pig-farmer. A hundred-box churn will deliver 700 gallons to 800 gallons of buttermilk within a few minutes. Storage space would be required to hold the volume of buttermilk necessary to keep the separator running continuously from the commencement of separation until completion of the buttermilk discharge from the day's churnings. In this respect the position is rather more inconvenient than for the separation of whey, since whey is usually discharged from a series of vats in close succession, whereas the discharge of buttermilk in a large butter-factory may extend over six to eight hours. The cost of provision of extra buttermilk-storage capacity must be added to the estimated cost of separation cited above. The whole question requires investigation under New Zealand conditions before any conclusions can be drawn as to the practicability of, and the economic returns likely to be obtained from, the separation of sweet-cream buttermilk in our factories.

#### MANUFACTURE OF CASEIN FROM BUTTERMILK

Buttermilk casein cannot be other than of inferior quality, on account of the high fat content (up to 10 per cent.). The high fat content of the casein greatly restricts the possible uses. Casein is made from buttermilk by one butter-manufacturing company in Australia, and the casein is mixed with lactic casein and sold for manufacture of glue according to a special formula. For the making of the normal casein glues, however, buttermilk casein has been found to serve as little more than a filler, for which purpose bentonite may more economically and more conveniently be used(17). Casein is difficult to prepare from buttermilk, as the curd does not settle readily and the water is not easily pressed out. A steam injection method of preparation of casein from buttermilk has been used in the United States(18). The American workers report that the buttermilk casein is inferior industrially to skim-milk casein, but that it can be used if mixed with skim-milk casein. They state that the drying of buttermilk casein is deleterious and should be avoided if possible, and they consider its most suitable sphere of usefulness is in the manufacture of water-paints. If the buttermilk casein were to be used without drying for water-paints, the factory making the paint would have to be relatively close to the factory producing the casein and the volume of paint manufactured would fluctuate with the seasonal fluctuations in casein-production. Buttermilk casein has been prepared commercially in New Zealand, but the price obtained from the buyers overseas was unremunerative and its manufacture was abandoned. On the whole, it does not seem that casein preparation from buttermilk is likely to be a promising outlet for the utilization of buttermilk, and the problem of disposal of the casein whey would still remain. It seems preferable to seek an outlet for the buttermilk where the fat content will be a help rather than a hindrance.



## CONDENSING OF BUTTERMILK

In the United States, Rogers and his co-workers(19) developed a method of preparing from buttermilk a condensed product called "semi-solid buttermilk" which would maintain its quality on keeping. The buttermilk is warmed to 100° F., and is then inoculated with a culture of a *streptococcus* and a *mycoderm* which, growing symbiotically, produce an acidity of 1.2 per cent. to 1.5 per cent. lactic acid in the buttermilk. This acid buttermilk is then condensed in a vacuum pan to approximately one-third of its volume, giving a semi-solid product. The product should have a fine-grain curd, and the final acidity should not be less than 4 per cent. lactic acid, which is sufficient to "pickle" the product—i.e., to prevent the growth of micro-organisms.

As an economic means for the disposal of buttermilk the process has several disadvantages: (a) the cost of floor space and of vats for holding the buttermilk during fermentation and of the condensing-pan capacity required in relation to the returns for the use of the product for animal feeding; (b) the packaging and transport costs of such a highly acid liquid product (the only suitable container is the wooden barrel); (c) the conversion of so much of the lactose to lactic acid reduces the feeding value of the buttermilk.

The composition of semi-solid buttermilk is given by Hunziker(20), p. 292) as—

					Per Cent.
Total solids content	..	..	..	..	25.00 - 32.70
Fat ..	..	..	..	..	1.00 - 2.60
Protein ..	..	..	..	..	9.00 - 11.85
Milk sugar ..	..	..	..	..	3.96 - 12.00
Ash ..	..	..	..	..	1.70 - 7.51
Lactic acid ..	..	..	..	..	4.30 - 6.19
Water ..	..	..	..	..	67.30 - 75.00

The degree of concentration obtained is not very high. For such a bulky product the cost of containers and of internal transport, added to the cost of production, render the process economically unattractive as an outlet for buttermilk.

Sweet-cream buttermilk can be condensed to a concentrated product suitable for direct use by bakers or in the manufacture of ice-cream. In this form it may be shipped in cans or returnable barrels, thus reducing the container cost.

The production of concentrated buttermilk products (as distinct from dried buttermilk) in the United States in 1943 was 73,600 tons. There is no record of the proportions of this quantity used for human food and for animal feeding respectively. The production of creamery butter in the United States in the same year was 746,900 tons. The weight of concentrated buttermilk products was thus approximately 10 per cent. of the weight of creamery butter made.

## DIRECT UTILIZATION OF BUTTERMILK FOR HUMAN CONSUMPTION

Buttermilk is an excellent food product and it is widely used for direct human consumption in liquid form. In many countries the buttermilk is sold in bottles by the milk-roundsmen. Capstick, for example(15), reports that an organization in Scotland (1937) was selling 1,700 gallons of liquid buttermilk daily, approximatey one-half in pint bottles for domestic use, and one-half in larger quantities for bakers. Some of this may have been buttermilk from the churning of milk for buttermaking. Such buttermilk

is undiluted and has a high fat content and an excellent flavour. Where a butter-factory is situated in or near a town, there seems to be a possible outlet for the direct sale of buttermilk to domestic consumers and to bakers. In Germany a certain amount of buttermilk is converted to a form of "cottage cheese." In New Zealand, however, cottage cheese is not a regular article of diet and any considerable utilization of buttermilk in this way could only be a gradual development.

Where buttermilk is to be used for human food, the system of collection from the churn would require some improvement over present methods. The buttermilk would have to be transferred direct from the churn chute through sanitary piping to a container vat.

#### DRYING OF BUTTERMILK

Buttermilk, especially the sweet-cream buttermilk obtained from the churning of daily collection cream, can readily be dried to powder form. According to the quality of the product, it may be used for human food or for animal-feeding purposes. In the United States in 1943 the production of dried buttermilk was 27,000 tons. During this year, as stated above, the butter-production was 746,900 tons. Hence, for each ton of butter made, 0.036 ton of dried buttermilk was produced. The buttermilk-production per ton of butter made is likely to be greater in the United States than in New Zealand on account of the lower average fat content of the cream; but, on the assumed value for New Zealand of 0.104 ton of buttermilk solids per ton of butter, it would appear that more than 30 per cent. of the buttermilk in the United States is converted to dry buttermilk. On the same percentage basis, New Zealand would produce between 3,000 tons and 4,000 tons of buttermilk powder.

#### *Method of Dry-buttermilk Production*

Buttermilk is not accumulated at a butter-factory in sufficient volume to maintain a spray-drying unit in continuous operation. The cost of a spray-drying unit would therefore be too great for installation solely for the drying of buttermilk, unless supplies from several factories were pooled. Some buttermilk powder is prepared in New Zealand by the spray process during the early and later months of the season when skim-milk supply is not adequate to keep all the drying units in continuous operation. For general butter-factory use, however, only drying by the roller process need be considered.

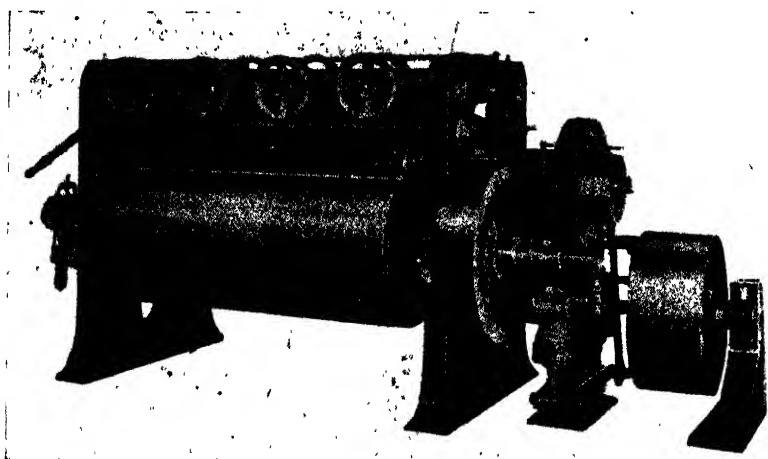
The buttermilk is run into the trough between two steam-heated hollow cylinders which are kept in constant rotation, one cylinder revolving rather more rapidly than the other (see Fig. 4). The distance between the cylinders is adjustable and is so regulated as to leave a film on the roll which will dry during the period of rotation. The dry film is removed from the cylinders by means of a scraper or "knife," falls into a screw conveyor, and is carried to a grinder and sieve. The grinder beaters may be adjusted to give the desired mesh size of the finished product. The sieved product is ready for immediate packaging. The production of a good-quality powder calls for a certain amount of skill and for continuous attention from the operator. The position of the knives relative to the cylinder surface must be carefully adjusted if, on the one hand, the film of dried milk is to be completely lifted from the roll, and if, on the other hand, the carefully machined surface of the cylinder is not to be scored and made uneven. Without careful adjustment the film may not be completely removed from the rolls, some browning

and burning of the residue will take place, and the powder will contain brown specks of burnt material. Such powder is suitable only for animal food. The knives are usually "touched up" daily by means of an oil-stone, and require regrinding after forty hours' running.

On the large scale it is more satisfactory to condense the buttermilk to a more concentrated product in a vacuum pan before drying on the rolls, but this is not necessarily so for a small plant as the cost of the condensing equipment is high. Drying of pre-condensed buttermilk gives a rather better product, as the film leaves the rollers more easily and there is less risk of burning the powder.

#### *Requirements for the Drying of Buttermilk Powder for Human Consumption*

The first requirement in the present-day butter-factory is a new system of collection of buttermilk from the churn to replace the present system of discharging to a sump via a floor drain. Some system of pumping from the churn chute through sanitary piping to an insulated storage vat is essential. This may slow down churning operations to a slight extent.



*Courtesy James Bell Machinery Pty., Ltd.*

FIG. 4.—A twin-roller drier suitable for use in the drying of buttermilk.

Storage vats capable of holding the greater portion of the day's supply of buttermilk are necessary. If the drying capacity of the plant is not sufficient to dispose of the buttermilk within a few hours of discharge from the churn, the vats should be insulated to prevent rise of temperature and consequent bacterial deterioration of the buttermilk during the period of storage. They may quite conveniently be placed in the butter-room if space is available. The milk-drying room is best situated away from the butter-room, and, if possible, in a separate building, as there is a certain amount of dust formation during the sieving of the powder. It is not desirable that this dust should be spread through the butter-factory, as it clings to ceilings, beams, and walls and encourages the growth of mould. Some of the modern grinding equipment attached to the drying rolls is designed to prevent the dissemination of dust during the packaging.

*Size of Plant required*

One of the chief difficulties in the economic production of dry buttermilk in New Zealand is the pronounced seasonal nature of the supply. A factory installing plant must choose between two alternatives :—

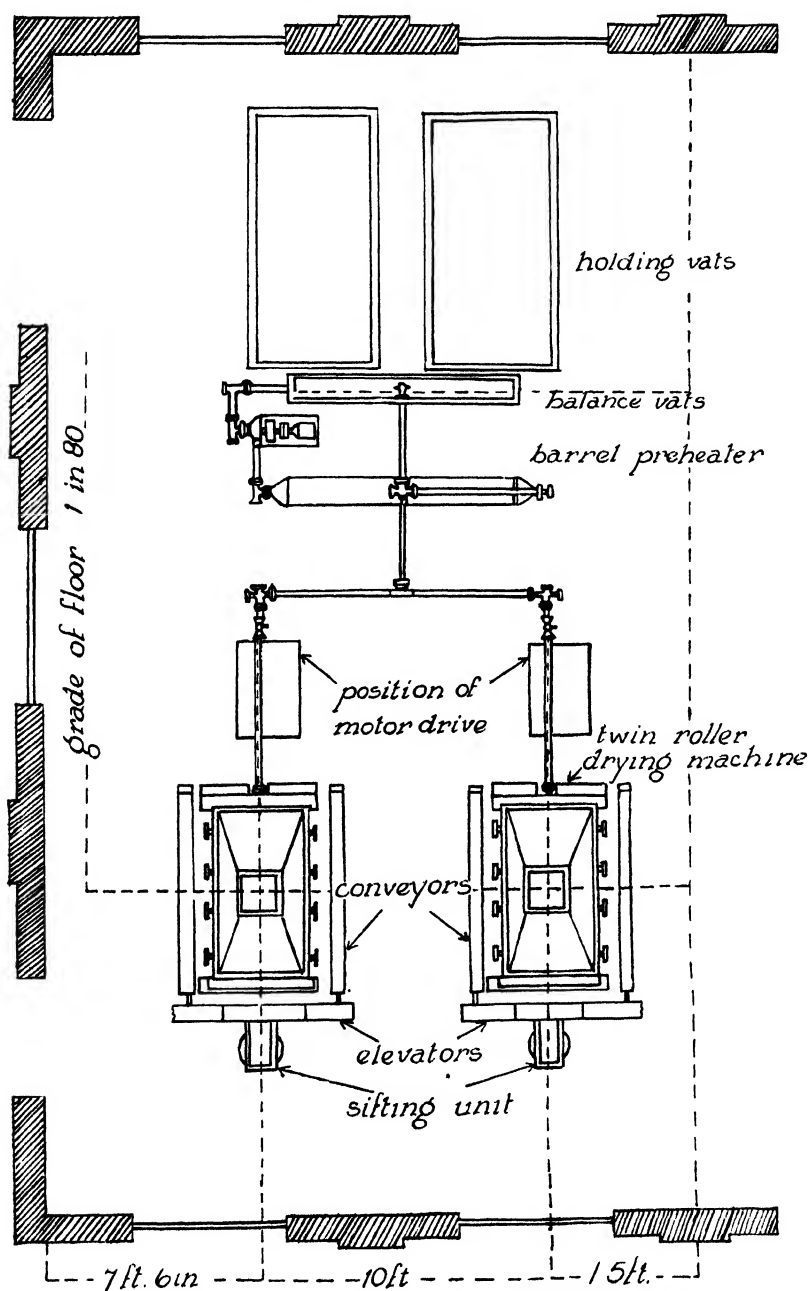
- (a) Installing sufficient plant to dry the peak production of buttermilk—the plant would then be running at full capacity for only a short period ; or
- (b) Installing sufficient plant to dry only a portion of the peak production, thus providing for the plant running at full capacity for a longer period.

Each of these alternatives has certain disadvantages :—

- (a) The cost of the installation is high relative to the yearly output. A normal-size twin-roll with preheater for warming the buttermilk before it reaches the drying roll, will dry about 100 gallons per hour. On the assumption that the plant can be kept running continuously for eighteen hours daily, the single roll will deal daily with 1,800 gallons of buttermilk. From the lines in Fig. 3 it can be seen that, for the average factory taking care to avoid excessive dilution of cream during processing, one set of rolls would dry all the buttermilk from a 1,500-ton factory except during three to four months of the year, when 300 gallons to 400 gallons of buttermilk would run to waste. If during this period the plant could be run for twenty-one to twenty-two hours daily, the whole of the buttermilk production could be dried on the one set of rolls. The plant would be running at full capacity for only these three to four months, unless skim-milk could be purchased to supplement the supply during the early and later months of the season. A two-roll plant would just dry all the buttermilk from a 2,500-ton factory, but from a 3,000-ton factory, up to 500 gallons of buttermilk would be run to waste during three to four months at the height of the season. A three-roll plant would just suffice to dry the buttermilk from a 4,000-ton factory. If running the plant at night is to be avoided, the size of the drying-plant would have to be correspondingly increased.
- (b) If the plant is installed to cope with only a portion of the supply, the excess supply becomes available at a time when pig feed is plentiful, and it is then difficult to dispose of. In most factories discharge into the drains would cause a serious drainage problem difficult to overcome.

*Space required for the Drying Equipment*

In the layout considered necessary for a two-roll plant, a space of 25 ft. × 25 ft. is allotted for the accommodation of the drying and sifting machinery. A three-roll plant would require an extra width of 10 ft. to 12 ft. (see Fig. 5), and a four-roll plant can be installed in a building 45 ft. wide. The length of 25 ft. would be sufficient for the drying and sifting machinery in all these sizes of plants, but extra length would be required for preheating or condensing plant if these are included, and for storage vats if these are not installed in the butter-room. The vat and preheater accommodation required calls for a further 15 ft. × 25 ft. of floor space,



Courtesy James Bell Machinery Pty., Ltd.

FIG. 5.—Layout suggested for a two-roll buttermilk-drying plant, including space for holding-vats and barrel preheater. If holding-vat space available in butter-room, area can be correspondingly reduced.

making, for a two-roll plant, a building measuring 25 ft.  $\times$  40 ft.\* In addition, a room for storage of containers and of buttermilk powder, as large as may be thought necessary according to the speed of disposal after production, is required. This room should be separated from the drying-room by a damp-proof wall, since buttermilk powder in sacks readily absorbs water from a damp atmosphere.

### *Boiler Capacity required*

The boiler capacity required depends to some extent on the system of drying adopted—*e.g.*, return of the hot condensate under pressure from the rolls to the boiler increases the steaming capacity of the boiler by 25 per cent. A single roll with belt drive, on steam-engine power, may require a 30 h.p. boiler for running at 100 gallons per hour. When electric-motor power is used, the boiler capacity required would be 15 h.p. With a pre-heater a somewhat greater boiler capacity is needed, but this is reflected in greater output.

The boiler capacity for operation of a roller drier is thus very considerable and few butter-factories will have this spare capacity during the daytime when vacreator treatment of the cream and reception of the cream and can-washing are proceeding simultaneously. After the completion of cream reception, by which time also the churns are cleaned and sterilized, spare steam from the boiler is most likely to be available. It is desirable that if the factory boiler is to be used there should be spare steam over and above that needed for treatment of the cream sufficient to maintain a boiler pressure of not less than 65 lb. per square inch to the drying unit. Otherwise in the flush months of the year the time of operation of the plant may be restricted by the necessity for extended treatment of the cream, or, alternatively the grade of the butter may suffer through undue speed of treatment in order to supply the demands of the drying plant for steam. The use of the butter-factory boiler has the further disadvantages that (a) the boiler would be running continuously day and night, and the necessary opportunity for blowing down and for cleaning out the grate or repairs to the fire-bars would be lacking. It is safe to say that with present-day fuels and working conditions a periodic break in the operation of the boiler would be necessary; and (b) the re-use of condensed steam from the drying rolls may lead to the introduction of off-flavours via the steam to the cream, and so affect the butter quality. The circuit for the return of condensed steam is however, totally enclosed, and if it is kept in good repair the danger of contamination of the steam should not be serious.

It is clear that for optimum service to butter-factory and drying unit a separate boiler is desirable, unless the plant size is arranged so that the drying can be completed and still leave the boiler free for a few hours periodically. For economy of labour during the operation of the plant at night it is desirable that the drying plant should not be placed at any great distance from the boiler-room. One operator can attend to as many as four drying rolls, but constant supervision is necessary, and any prolonged absence for attention to the boiler may lead to the production of second-grade powder. The installation of a separate boiler would make it possible to run the drying plant during the flush months for a longer period than eighteen hours and so increase the ability of the plant to dispose of the flush supply.

\* These dimensions would provide ample room. Where necessary to fit into space already available, the plant could probably be installed in less space than that set out above.

### *Packaging of Product*

For local sale for human food or for animal food the buttermilk powder may be packed in hessian bags with paper liners. In Australia, liners of water-proof "Sisalkraft" are used for powder for human consumption, and paper liners for powder for animal feed. The powder is very bulky. The 84 lb. packed in each bag occupy 5 cubic feet, and the bags used are 40 in. long  $\times$  17½ in. wide.

For export, the "Sisalkraft" liners may be satisfactory, but it seems possible that packaging in tins may be necessary, as for whole-milk and skim-milk powder.

### *Composition and Food Value of Dried Buttermilk*

Dried buttermilk contains a higher proportion of fat than dried skim-milk. The actual fat content depends on the fat losses in churning. As, however, the butterfat is more valuable in butter than in dried buttermilk, the aim would naturally be to maintain as low a fat loss as possible. With a fat content of 0.8 per cent. in the buttermilk, the percentage of fat in the dried product would be 10 per cent., compared with 1 per cent. to 1.5 per cent. for skim-milk powder. The extra fat content renders the product rather more valuable as a human food. Where, under recent legislation, city milk-prices have to be paid for butterfat for ice-cream manufacture, the fat in dry buttermilk is a valuable constituent. Thomas and Combs(21) found that sweet-cream roller-process buttermilk powder gave better whipping properties to an ice-cream mix than skim-milk powder, and the resultant product was more stable. However, the outlet for dry buttermilk for manufacture of ice-cream in New Zealand is limited and is variable according to the climatic conditions. Fresh buttermilk powder should be equally as good as skim-milk powder for use in bread.

The composition of buttermilk powder is likely to be variable, as already stated. The figures given in Table XII represent an average analysis. Corresponding figures for an average meat meal are given for comparison.

TABLE XII.—COMPOSITION OF AN AVERAGE BUTTERMILK POWDER AND A GOOD-QUALITY MEAT MEAL

Constituent.		Buttermilk.	Meat Meal.
		Per Cent.	Per Cent.
Moisture	..	4.0	10.0
Fat	..	10.0	18.0
Protein	..	35.0	67.0
Lactose	..	42.2	..
Acidity	..	0.8	..
Ash	..	8.0	5.0

The moisture content of buttermilk powder is usually somewhat higher than that of skim-milk powder, as buttermilk powder is more hygroscopic, but the general rule holds that the higher the moisture content, the lower the keeping quality of the powder. The milk powder from neutralized-cream buttermilk also takes up moisture more readily than powder from

sweet-cream buttermilk. The high content of fat renders the dried buttermilk more susceptible to decomposition during storage than dried skim-milk. This is accentuated by the conditions of pre-treatment of buttermilk as compared with skim-milk, which is usually delivered twice daily for drying. The temperature of storage of the powder has an important effect on the keeping quality. Davis(22) found that even buttermilk powders from sweet cream deteriorated very seriously in flavour and colour when held in sealed containers for one year at room temperature, and the flavour deterioration was still very marked when the samples were stored in a refrigerator at 35° F. to 50° F. Buttermilk powder for human consumption is therefore a product which should have a fairly steady and consistent outlet if there are not to be losses through deterioration in quality. There is no reliable evidence as to the length of time dried buttermilk from sweet cream will remain in edible condition. For any one sample this time will vary with the temperature of storage and the type of packaging, and there would be variations according to the quality of powder itself at time of manufacture. Poor-quality dried buttermilk has a distinctly objectionable flavour, and the delivery of old and deteriorated powder may spoil the market for good-quality powder. Dried buttermilk will keep in a condition suitable for use as animal food over a considerable period of time if the storage conditions and packaging are satisfactory, but stale buttermilk powder should be kept well away from the butter-room. Buttermilk powder kept in sacks tends to cake and harden, and this may cause inconvenience in the use of the powder for animal food. Such hardened powder may require grinding and sieving before it can be used. Some users of buttermilk powder for poultry feeding make a practice of transferring the powder from the sacks to airtight tins immediately after receipt.

#### *Disposal of Buttermilk Powder*

(a) *The Export Market.*—The future market position for all dried-milk products is uncertain and it is not possible to provide any very reliable guide to the situation. There is at present, during the period of world food stringency, a ready demand for all types of food, but the world production of dried milk has been greatly increased during the war years and it is difficult to foresee how far this increased production will eventually overload the civilian market. Leonard(23) reports, for example, that the post-war production capacity of the American dry-milk industry is 1,250,000,000 lb. (560,000 tons) and that the domestic market had consumed in pre-war years only about 450,000,000 lb. (200,000 tons). Leonard states that the excess is available for export and that plans were being made for the development of the export market. Dried-milk production has increased also in other countries, particularly Australia, Canada, and Great Britain, but the figures are not at present available. Of the skim-milk powder sold in United States, Leonard reports that bakers take 55 per cent., ice-cream manufacturers 14 per cent., and the meat industry 6 per cent. If dried milk can be sold on the Eastern market, the absorption of the present world supply would be assured and there would be room for further expansion of production in New Zealand.



Dried buttermilk is a less suitable product for export than skim-milk powder. If a wider export market for skim-milk powder does not develop, however, the sale of buttermilk powder in New Zealand for human food will have to meet the competition of skim-milk powder, and it is difficult to estimate how much will be easily saleable. There is apparently a market in Australia for buttermilk powder at £35 to £40 per ton f.o.r. Melbourne, but the present indications are that packaging in tins might be necessary. Also, during the war and the present food emergency much of Australia's skim-milk powder has been commandeered for export and the demand for the powder locally in Australia has exceeded the supply. When this position is changed there may be less demand for buttermilk powder. If, however, buttermilk powder is preferred to skim-milk powder on account of its higher fat content, the quality of powder obtained from New Zealand sweet cream should be eminently suitable for the market. The keeping quality of buttermilk powder under export conditions of transport requires careful exploration.

(b) *The Local Market.*—The consumption of concentrated milk products in New Zealand in the year 1945 was as follows:—

					Tons.
Whole-milk powder	..	..	..	..	800
Skim-milk powder	..	..	..	..	2,300
Evaporated milk (unsweetened)	..	..	..	..	200
Condensed milk	..	..	..	..	1,800

These figures are probably higher than the normal peacetime consumption owing to the use of the powders for the making of Army biscuits. The total yearly production of milk powder in New Zealand at the present time is about 10,000 tons. It is clear, therefore, that the amount of dried buttermilk that may be able to edge its way into the present market is not very large. A 2,000-ton butter-factory will produce roughly 200 tons, of which about 150 tons might be suitable for human consumption. There is the possibility that dried buttermilk may find a market other than the present market provides. At the present time the Wheat Research Institute are comparing the use of dried buttermilk and dried skim-milk as an addition to bread. If New Zealand used dried milk in bread in about the same proportion as in the United States, the amount used in this way would be about 1,800 tons. No doubt a considerable proportion of the 1,800 tons of skim-milk powder used in New Zealand is used in breadmaking, but there is no available indication of the exact quantity so used. The total amount of flour used for all purposes by bakers in New Zealand is 107,000 tons. If 4 per cent. of milk powder were used in all this flour, the total consumption for breadmaking would be 4,280 tons.

Condensed milk or milk powder is required for ice-cream making. The quantity of milk powder used for ice-cream in New Zealand is 300 tons to 400 tons. Other things being equal, buttermilk powder should command a premium over skim-milk powder for this purpose on account of the higher butterfat content. One pound of buttermilk powder will contain 0.1 lb. of butterfat, which has to be purchased by ice-cream makers in city areas at city milk-prices. The value of this butterfat to them is, at their prices, not less than 2d. per pound of milk powder. The size and steadiness of the market is, however, all-important on account of the effect of age, and especially of high storage temperatures, on the quality of the powder. With a break in the steady stream of disposal, loss may be incurred through deterioration of the state of powder from human-food to animal-food quality.

## UTILIZATION FOR ANIMAL FEEDING

There is a market for a certain amount of buttermilk powder for animal-feeding purposes—e.g., for inclusion in poultry rations, for calf feeding, and possibly to some extent for pig feeding. Capstick(15) reports that most of the dried buttermilk made in England is used for inclusion in cattle foods.

(a) *Poultry Feeding*.—The inclusion of some milk powder in a poultry ration appears to have a beneficial effect on the health of the poultry, although Hart and Stuart(24) in a comparison with a non-milk-containing basal ration found only slight increases in growth and egg-production, not sufficient to cover the extra cost of the milk product. Many commercial poultry-keepers add 5 per cent. of milk powder in the mash, and up to 12 per cent. in the mash for the raising of chickens. In Australia and the United States many of the ready prepared poultry mashes contain buttermilk powder. The total number of fowls in New Zealand is 4,000,000 and the consumption of mash per fowl may be taken as 40 lb. On the calculated addition of 5 per cent. of milk powder to the mash for all the fowls in the country, the total requirement in milk powder would be 8,000,000 lb.—i.e., roughly 3,500 tons. Many of the fowls, however, are kept on farms with access to liquid skim-milk, and it would not be expected that the use of the powder would be universal even among commercial poultry-keepers. It should be possible, however, to increase the utilization of milk powder for poultry feeding to as much as 1,000 tons yearly if the price were not too high. It is not possible to estimate the immediate probable consumption.

(b) *Calf Feeding*.—It is well known that skim-milk can be used for the feeding of calves after they have been given whole milk for a few weeks. This skim-milk is already available on the majority of New Zealand dairy-farms—viz., those supplying butter-factories—and there would be no call for buttermilk powder for calf feeding from butter-factory suppliers. Cheese-factory suppliers have no skim-milk available on the farms, but a number use whey with meat meal or some other calf meal as a substitute. If the price is not too high, there may eventually be a demand from cheese-factory suppliers for buttermilk powder for calf feeding. Suppliers to cheese-factories selling the whey for lactose-manufacture are likely to use whey mother liquor in preference to dried buttermilk. The suppliers to dried-milk factories and to condenseries have no dairy by-products left on the farm, and, in the absence of any powder of second-grade quality from their own factories, are likely to use a certain amount of buttermilk powder for calf feeding. For the feeding of all calves on farms supplying milk-concentrating plants for three months at the rate of 1 lb. to 1½ lb. of powder daily (equivalent to 1 gallon to 1½ gallons of skim-milk) the total buttermilk powder requirements would be of the order of 400 tons to 500 tons annually. It seems more likely, however, that the cheaper meals now in common use would not be wholly displaced by dried buttermilk, and the amount likely to be called for from suppliers to dried-milk factories would not be more than 200 tons to 300 tons. There should also be a demand from suppliers of the local liquid-milk market, especially during the autumn and winter months when milk is in short supply, and a surplus is not likely to be available on the farms for separation. Details of the number of cows calving during the period March to August for supply to the local milk market are not available, but from figures of milk suppliers to the Auckland district, as quoted by the Milk Commission, it may be estimated (roughly) that the number would not be greater than twenty thousand to thirty thousand, and the call for butter-milk powder for calf feeding for these herds would not be greater than 150 tons.

The probable total demand for calf feeding is thus not likely to exceed 450 tons, and the maximum demand for this purpose would appear to be in the region of 800 tons. As stated earlier, the demand would be related to the price of the powder in comparison with the price of other sources of feed. Buttermilk powder at £25 per ton would be equivalent to about 2·4d. per gallon of skim-milk.

The demand for calf feeding would be greatest in August, September, and October. The production during these months is likely to be of poorer quality suitable for animal feeding. If, however, the total production for the year was suitable only for animal feeding, the greatest volume of production—i.e., in November, December, and January—would occur at the time of lowest demand, and the time of longest storage would coincide with the time of highest storage temperatures.

(c) *Pig Feeding.*—The demand for dried buttermilk for the feeding of pigs would depend on the price of pig meat and on the availability of meat meal. The cost of production of dried buttermilk, as shown later, is fairly static, independent of the prevailing standard of prices, whereas the price of pig meat may be such that it is not possible to recover the cost of buttermilk powder purchased at this price for pig feeding. Meat meal which has a higher protein content than dried buttermilk, and may be said to be a slightly more concentrated animal food, is usually sold at little more than half the cost of dried buttermilk. For pig feeding, therefore, dried buttermilk is likely to prove much less attractive than meat meal. There is no means for estimating the likely demand for dried buttermilk for pig feeding.

The likely demand for dried buttermilk for poultry, pig, and calf feeding in New Zealand is thus difficult to assess, and no very reliable estimate can be given to dairy companies considering the installation of a buttermilk-drying plant. It is possible that there may be an overseas market for the product for animal-feeding purposes—e.g., for calf feeding in Great Britain, where the greatest proportion of the milk is used for the liquid-milk market. The whole question of packaging for export, however, requires some clarification. Without a suitable waterproof package, dried buttermilk would cake and deteriorate in flavour under the warm humid conditions in the Tropics.

#### YIELD OF BUTTERMILK POWDER

In the production of buttermilk powder a certain amount of loss takes place through spillage of the liquid buttermilk and through wastage of the finished product. On the other hand, the powder as produced contains 3 per cent. to 5 per cent. of water. On the assumed basis of 0·104 ton of buttermilk solids in the buttermilk discharged per ton of butter, it could be expected that the buttermilk powder recovered would be of the order of 0·10 ton per ton of butter, or 10 per cent. of the weight of butter churned.

#### *Cost of the Installation for Drying Buttermilk*

It is not possible to present any universally applicable statement on the cost of an installation for the drying of buttermilk. The cost will vary according to the layout adopted, the distance of the drying-room from the butter-room, the type of building erected, the type of vat used for holding the buttermilk, the measures required to be taken to provide the boiler capacity, and a number of other factors. From the information available, however, it is possible to give some indication for a plant of a postulated

size. For purposes of this discussion, a two-roll plant will be taken and the prices will be those quoted by a well-known supply house for present delivery (Table XIII).

TABLE XIII.—APPROXIMATE ESTIMATED COST OF A TWO-ROLL DRYING UNIT

Building, 25 ft. × 40 ft.— <i>i.e.</i> , sufficient to house holding vat, preheater, and drying units, but not for storage of powder: 1,000 square feet, say	£	s.	d.
Two twin-roller drying units with electric drive, at £1,020 each ..	1,500	0	0
One electrically driven knife grinder .. .. .	2,040	0	0
One sifter and grinder and motor drive .. .. .	140	0	0
One preheater .. .. .	85	0	0
One motor-driven milk-pump .. .. .	100	0	0
Two cowls and ventilator shafts .. .. .	35	0	0
Two sets bins .. .. .	240	0	0
Collector trays for collection of buttermilk from churns, with large-capacity pump and motor and starter and sanitary tubing, taps, unions, &c., say .. .. .	55	0	0
One holder vat for buttermilk, say .. .. .	300	0	0
Foundations, piping, fittings, say .. .. .	300	0	0
Electrical installation, say .. .. .	200	0	0
Labour of installation: Two men for eight weeks .. .. .	150	0	0
	280	0	0
	<hr/> £5,425 10 0 <hr/>		

It seems probable from the above schedule that, with various secondary costs such as railage, cartage, and sales-tax, a two-roll plant would cost not less than £6,000 without allowance for cost of the boiler. If a special boiler, stoker, and boiler housing were required, the total extra cost would be about £1,500, making a total cost of £7,500.

A two-roll plant would produce from a 2,500-ton butter-factory about 220 tons of dried buttermilk (allowing for some wastage during the flush months). On an installation cost of £7,500, allowance for interest at 5 per cent. and for depreciation and maintenance at 10 per cent., would amount to £1,125 annually, or roughly £5 per ton of dried product.

### *Working Costs*

The working costs are difficult to assess with any certainty, on account of the variability of the labour costs. A two-roll plant making dried milk for human food should be under fairly constant supervision, and this would only be possible if an operator were detailed to the work. With two sets of rolls running for nineteen hours per day and drying 100 gallons per hour, the maximum total production, if every care were taken in the butter-factory to reduce dilution, would be 1 ton 7 cwt. of powder. With allowance for three men each working eight hours daily to cover the drying and cleaning, the wages costs for operation alone would be about £3 10s. per day, without allowances for cartage, overhead administration, and other sundry costs. During the early and later portions of the season, also, the production per roll would be much smaller, giving higher wages costs per ton of powder. For all these working costs, therefore, it seems necessary to allow as a minimum £5 per ton of powder.

### *Fuel and Power Costs*

The fuel requirements for running a roller drier vary with the system adopted—*e.g.*, a pressure pump returning condensate from the rolls to the boiler effects a very considerable reduction in fuel required. For use of steam for drying and other purposes in the plant the steam requirement is not likely to be lower than  $1\frac{1}{2}$  lb. of steam to evaporate 1 lb. of water from the buttermilk. On this basis and with allowance of 5 lb. of steam produced from 1 lb. of coal, it would take 2.7 tons of coal to produce 1 ton of powder from buttermilk containing 8.5 per cent. of total solids, 2.9 tons from buttermilk containing 8.0 per cent. total solids, and 3.2 tons from buttermilk containing 7.5 per cent. of total solids.

The cost of fuel naturally varies with the locality of the plant, due to variation in transport costs. At £2 per ton for fuel at the bunker, the fuel costs become £5 8s. to £6 8s. per ton of powder according to the total solids content of the buttermilk.

The power requirements for a normal twin-roll with pumps and grinder, all electrically driven, are approximately 15 h.p. Taken at 10 units of power per hour at 1d. per unit, the cost for power becomes 10d. for the powder from 100 gallons of buttermilk—*i.e.*, for 85 lb. of dried product—or 22s. per ton.

### *Cost of Packaging*

Dried buttermilk is a bulky article and it easily absorbs water vapour. For use as an animal food it may be packaged in paper-lined sacks. For use as human food, if consumption is not unduly delayed, the same type of package may be used; but for export under the humid conditions of sea transport it may be found necessary to package in tin containers. The packaging costs would thus depend on the destination of the product. Packaging in paper-lined sacks would cost approximately £4 per ton of powder.

### *Summary of Estimated Costs of Production\**

The costs of production per ton of powder, as estimated above, may now be listed :—

					£	s.	d.
Interest and depreciation	..	..	..	..	5	0	0
Labour	..	..	..	..	5	0	0
Fuel	..	..	..	..	6	0	0
Power	..	..	..	..	1	2	0
Packaging	..	..	..	..	4	0	0
					£21	2	0

The actual cost of producing the powder would thus appear to be in the neighbourhood of £21 per ton at the factory, without allowance for any value for the buttermilk, and assuming that there is very little dilution of the buttermilk during processing at the factory. In the early and later

\* These estimated costs may be compared with those quoted by Hunziker(20) for American conditions on the basis of production of 70 tons of finished product—Steam : 1,500 lb. to give 100 lb. of powder. At 5 lb. steam per pound of coal, this is equivalent to 3 tons of coal per ton of powder. Power :  $6\frac{1}{2}$  kWh. per 100 lb. of powder, or 150 units per ton. Labour :  $\frac{1}{2}$  hour per 100 lb. of powder, or 11.2 hours per ton of powder. Depreciation : 10 per cent. Interest : 6 per cent.

months of the season, when water may be added with the steam for washing out of cans and when the rinsings form a larger proportion of the total volume of cream, the cost would undoubtedly be higher per ton of powder owing to the increased dilution.

The present value of buttermilk, on the average for all factories in New Zealand, is 2s. 10½d. per ton of butter or £1 10s. per ton of dried buttermilk. For factories owning their own pig-farms the average return for buttermilk was 5s. per ton of butter, equivalent to £2 10s. per ton of dried buttermilk. If the above estimates are sound for any one factory, it would then be necessary for the factory to receive an average of at least £23 10s. per ton of buttermilk to equal the present returns from direct disposal of buttermilk without drying. The present margin for sales costs of buttermilk powder is £5 per ton, which would bring the retail price to £28 10s. per ton. The present retail price of the powder for animal-feeding purposes in New Zealand is 3d. per pound, or £28 per ton.

### *Discussion and Summary*

It seems clear that if buttermilk powder can be sold for human food at not less than £30 per ton at the factory, the drying of buttermilk should be profitable. A steady demand is desirable to eliminate long storage, which may cause deterioration from human-food to animal-food quality. In any plant the production of a proportion of the output as animal-food quality can scarcely be avoided.

The extent of the present market for dried buttermilk for use as human food is uncertain. The total local consumption of dried-milk products does not offer any very extensive scope for the expansion of dried-buttermilk production, but it may be possible to expand the market. Dried buttermilk from sweet cream has the advantage over skim-milk powder that it has a higher fat content, and this may affect the relative demand for the two products.

The cost of producing dried buttermilk will vary with the size of the plant, the system adopted, the location of the plant, and the degree of dilution of the buttermilk with water, but as a rough guide it appears that, for the drying of buttermilk for animal feeding, a price of at least £23 10s. would be required at the factory to equal the present returns from the sale of liquid buttermilk. Sale of a certain amount of dried buttermilk at this price, plus selling charges, for the feeding of calves and poultry seems likely. It is uncertain how much demand there would be for dried buttermilk for the feeding of pigs.

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## STUDIES ON THE NEUTRALIZATION OF CREAM FOR BUTTERMAKING

### VII. NEUTRALIZATION IN PRACTICE IN THE BUTTER-FACTORY

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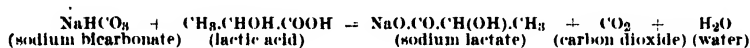
#### Summary

Owing to the effect of carbon dioxide on the initial acidity titre of cream, the theoretical factor (the amount of lactic acid neutralized by 1 lb. of sodium bicarbonate)—viz., 1.07—for calculation of quantity of sodium bicarbonate neutralizer to be added is too low. The Valentine factor (1.2) is sound for low-acid creams, but too high for high-acid creams. A chart is presented based on a graduated transition from the factor 1.2 to the factor 1.10 as the degree of acidity reduction required is increased. Where measurement of cream samples for acidity titration is by volume, calibration of the neutralizing vat should also be by volume.

THE neutralization of cream for buttermaking has already been discussed from a number of the more theoretical aspects (1, 2, 3, 4, 5, 6). The present and concluding paper in the series deals with the application of the principles previously discussed, in butter-factory practice.

## NEUTRALIZATION CHART

It has been shown(5, 7) that the carbon dioxide present in the cream when received affects the acidity of the cream in the titration with alkali before neutralization, but does not absorb any neutralizer as it is lost along with the carbon dioxide formed from the sodium bicarbonate during the pasteurization of the cream. Flash-pasteurization of fresh cream without a neutralizer added results in a reduction in the acidity of the cream due to loss of carbon dioxide. It has been pointed out also that the Valentine factor for calculation of quantity of sodium bicarbonate to be added to cream for any desired acidity reduction(8) is at variance with the factor calculated from the equation—



According to this equation, 1 lb. of sodium bicarbonate should neutralize 1.07 lb. of lactic acid, whereas the Valentine factor, based on trials of cream neutralization in butter-factories, allows a neutralizing capacity of 1.2 lb. of lactic acid per pound of sodium bicarbonate used. The 12 per cent. variation of the Valentine factor from the theoretical has been shown to be due to the effect of the loss of carbon dioxide originally present in the cream in giving a reduction in acidity without absorbing neutralizer. The Valentine factor gives accurate results for the neutralization of sweet cream as the acidity due to carbon dioxide in the cream represents an appreciable proportion of the total acidity of the cream. When the cream is of high acidity, the carbon dioxide content is also higher than for fresh cream, due to the production of carbon dioxide by the lactic-acid bacteria (see (3), p. 821; (9) p. 147), but generally the proportion of the acidity titre due to free carbon dioxide becomes lower the higher the initial acidity of the cream. There is not, therefore, any single factor for calculation of quantity of neutralizer to be added which is valid for all types of cream. In Table I an attempt has been made to provide a chart which will make a graduated allowance for the effect of carbon dioxide in the cream on the acidity titre of the cream and therefore on the calculation of the quantity of neutralizer to be added.

It is assumed that the final acidity desired is 0.08–0.10 per cent. of lactic acid.\* For acidity reductions of up to 0.12 per cent. lactic acid the Valentine factor is used. Thereafter a gradual change of the factor is introduced, with increase in desired acidity reduction, until at a desired acidity reduction of above 0.24 per cent. lactic acid the factor is not varied from 1.10. This final 3 per cent. variation from the theoretical corresponds fairly closely with the effect of the increased carbon dioxide content of high-acid creams on the acidity titre. The chart has been tried out in a number of factories using flash-pasteurizers and has been found to give reasonably uniform results. On account of the natural variations in the carbon dioxide content of creams of any one acidity a neutralization chart accurately applicable to all creams is not obtainable.

\* If the final acidity desired is very low, 0.01–0.04 per cent. lactic acid, the carbon dioxide is not completely driven off during pasteurization.



TABLE 1.—NEUTRALIZATION CHART FOR SODIUM BICARBONATE ADDITION TO CREAM

(Table shows quantity of sodium bicarbonate neutralizer (pounds) to be added for different quantities of cream, from 50 gallons to 200 gallons in steps of 10 gallons, and from 200 gallons to 500 gallons in steps of 100 gallons)

Quantity of Cream	Percentage Acidity Reduction required.																		
	Factor.																		
	0.02	0.04	0.06	0.08	0.10	0.12	0.14	0.16	0.18	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.34	0.36	0.38
50	0.17	0.25	0.33	0.40	0.42	0.50	0.59	0.68	0.78	0.88	0.98	1.08	1.18	1.27	1.36	1.45	1.55	1.64	1.73
60	0.10	0.20	0.30	0.40	0.50	0.60	0.71	0.81	0.93	1.05	1.18	1.31	1.42	1.53	1.64	1.75	1.86	1.96	2.07
70	0.12	0.23	0.35	0.47	0.58	0.70	0.82	0.95	1.09	1.23	1.38	1.53	1.66	1.78	1.91	2.04	2.16	2.29	2.42
80	0.13	0.27	0.40	0.53	0.67	0.80	0.94	1.09	1.24	1.40	1.57	1.75	1.89	2.04	2.18	2.33	2.47	2.62	2.76
90	0.15	0.30	0.45	0.60	0.75	0.90	1.06	1.22	1.40	1.58	1.77	1.96	2.13	2.29	2.46	2.62	2.78	2.95	3.11
100	0.17	0.33	0.50	0.67	0.83	1.00	1.18	1.36	1.55	1.75	1.96	2.18	2.36	2.55	2.73	2.91	3.09	3.27	3.46
110	0.18	0.37	0.55	0.73	0.92	1.10	1.29	1.49	1.71	1.93	2.16	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80
120	0.20	0.40	0.60	0.80	1.00	1.20	1.41	1.63	1.86	2.11	2.36	2.62	2.84	3.06	3.27	3.49	3.71	3.93	4.15
130	0.22	0.43	0.65	0.87	1.08	1.30	1.53	1.76	2.02	2.28	2.55	2.84	3.07	3.31	3.55	3.78	4.02	4.26	4.49
140	0.23	0.47	0.70	0.93	1.17	1.40	1.65	1.90	2.17	2.46	2.75	3.06	3.31	3.56	3.82	4.07	4.33	4.58	4.84
150	0.25	0.50	0.75	1.00	1.25	1.50	1.76	2.03	2.33	2.63	2.95	3.27	3.55	3.82	4.09	4.36	4.64	4.91	5.18
160	0.27	0.53	0.80	1.07	1.33	1.60	1.88	2.17	2.48	2.81	3.14	3.49	3.78	4.07	4.36	4.66	4.95	5.24	5.53
170	0.28	0.57	0.85	1.13	1.43	1.70	2.00	2.31	2.64	2.98	3.34	3.71	4.01	4.33	4.64	4.95	5.26	5.56	5.87
180	0.30	0.60	0.90	1.20	1.50	1.80	2.12	2.44	2.79	3.16	3.54	3.93	4.26	4.58	4.91	5.24	5.56	5.89	6.20
190	0.32	0.63	0.95	1.27	1.58	1.90	2.24	2.58	2.95	3.33	3.73	4.15	4.49	4.84	5.18	5.53	5.87	6.20	6.56
200	0.33	0.67	1.00	1.33	1.67	2.00	2.35	2.71	3.10	3.51	3.93	4.36	4.73	5.09	5.46	5.82	6.18	6.55	6.91
300	0.50	1.00	1.50	2.00	2.50	3.00	3.53	4.07	4.67	5.26	5.89	6.55	7.09	7.64	8.18	8.73	9.27	9.82	10.36
400	0.67	1.33	2.00	2.67	3.33	4.00	4.71	5.42	6.21	7.02	7.86	8.73	9.46	10.10	10.71	11.32	11.93	12.54	13.15
500	0.83	1.67	2.50	3.33	4.17	5.00	5.88	6.78	7.76	8.77	9.82	10.91	11.82	12.73	13.64	14.55	15.46	16.36	17.27

Conversion of Fractions of 1 lb. to Ounces

Fractions of 1 lb...	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95
Ounces	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

## BOILING OF CREAM BEFORE ESTIMATION OF ACIDITY

The effect of carbon dioxide on the initial acidity titre can be eliminated if the sample of cream after being measured for the acidity titration is first boiled for a short time, as recommended by Newman and Wiley(9). This procedure is troublesome in factory practice, and with acid cream causes a curdling of the cream, with the result that the end-point colour differs considerably from the end-point colour obtained with uncurdled cream. When the cream is boiled before titration the factor used for calculation of amount of sodium bicarbonate to be added should be the theoretical value—viz., 1 lb. of sodium bicarbonate per 1.07 lb. of lactic acid. In practice, except with very gassy creams of high acidity, a type seldom encountered in New Zealand in any quantity, the allowance made in the chart will be found to give a sufficiently accurate assessment of the effect of the carbon dioxide present, especially if the acidity aimed at is 0.08-0.10 per cent., since a slight variation from the desired acidity within this range is of little consequence to the quality of the butter.

## ADDITION OF NEUTRALIZER TO VAT

(a) *Preparation of Sodium Bicarbonate Stock Solution.*—The quantity of neutralizer to be added should be measured with care. It may either be weighed in solid form, and dissolved in twenty times its weight of water before addition to the cream or, alternatively, a large batch of stock solution may be prepared in diluted form. It is not possible to prepare a strong solution of sodium bicarbonate on account of the low solubility in water—i.e., 9.6 parts in 100 parts of water at 68° F. If the water is warmed to 120° F. a solution containing 10 lb. of sodium bicarbonate in 90 lb. of water, or multiples of these amounts, can be prepared, and the bicarbonate will remain in solution at ordinary summer temperatures. With this stock solution the weight required will be that shown in the chart, multiplied by 10. The stock solution should be mixed with an equal volume of water before addition to the cream.

(b) *Use of Sodium-bicarbonate - Sodium-carbonate Mixtures.*—The use of sodium bicarbonate with high-acid cream results in the liberation of large amounts of carbon dioxide which may cause trouble from excessive frothing in the neutralizing-vat, and which may lower the vacuum obtainable in the vacreator. With cream of this type it is preferable to use a mixture of sodium carbonate and sodium bicarbonate. Prepared mixtures, or double compounds, of these neutralizers are available as sodium sesqui-carbonates, but the charts supplied with them by the manufacturers are usually based on the theoretical amounts required according to the chemical equation for the action of the neutralizer on lactic acid, and do not make any allowance for carbon dioxide content of the fresh cream. In preference to the use of special neutralizer mixtures it will be found less expensive and equally satisfactory to prepare a stock solution from sodium bicarbonate and soda ash. Four pounds of soda ash and 4 lb. of sodium bicarbonate added to 103 lb. of water will give a stock solution of the same neutralizing capacity as the above bicarbonate stock solution, and the weight required can be read from the chart by shifting the decimal point one place to the right. This stock solution also should be mixed with an equal volume of water before addition to the vat of cream.

(c) *Method of Addition of Neutralizer to Vat.*—In New Zealand, where the cream is mostly of low acidity, it is customary to add the neutralizer solution from a bucket or by means of a spray while the cream is being stirred. The stirring should be vigorous in order to avoid local concentration of neutralizer in contact with the cream. Hunziker recommends adding the neutralizer through a watering-can sprinkler. In Australia, where the quantities of neutralizers required are normally much greater than in New Zealand, various devices have been fitted to the neutralizing-vats to give a slow addition of neutralizer solution—*e.g.*, the neutralizer is added to a trough which extends along the length of the neutralizing-vat and is allowed to flow out slowly through holes in the bottom, the cream being stirred during the addition, or a container is fitted at the side of the neutralizing-vats with pipe connections and taps to a perforated pipe along the length of each vat. In some factories the water and solid neutralizer are added to the container and solution hastened by injection of steam. The neutralizer solution, however, is best added cold to the cream. Heating of sodium bicarbonate converts it to sodium carbonate.

(d) *Mechanical Stirrers in the Neutralizing-vats.*—Some form of mechanical stirrer is an advantage as it aids the liberation of carbon dioxide. If the cream is of very high acidity, however, the loss may be accompanied by production of so much froth that the cream flows over the top of the vat. The coil-type neutralizing-vats in use in Australia give excellent stirring of the cream, but if used to heat high-acid cream to which neutralizer has been added may also cause trouble through the production of too much froth.

#### DESIGN OF NEUTRALIZING-VAT

The neutralizing-vat is generally of a long, narrow shape, in order to give capacity without taking up too much space near the stage. When the cream is run from a tipping-vat by gravity, or is pumped through a header-line of pipes, this point is of little importance, and the design of vat may be arranged to suit the space available. It is important, however, that the vat should have a good slope to the outlet, and that the outlet should be of the well type. In the churning of cream one of the most important causes of increase of fat loss in the buttermilk is dilution of the cream in processing, and some dilution always takes place in the washing of froth from the neutralizing-vats. If the outlet is unsatisfactory, the quantity of water required is unnecessarily large. The quantity of water used is influenced by the type of pump used for drawing cream from the neutralizing-vat. A centrifugal pump has the disadvantage that it will not suck air, and is inefficient in the removal of froth from the vats. Some type of positive pump is more suitable for the purpose.

In Fig. 1 four typical forms of outlet from a neutralizing-vat are shown. Type A, in which the bottom of the outlet is flush with the bottom of the vat, is entirely unsuitable, as the pump begins to suck air as soon as the level of the cream reaches the top of the outlet. Type B, in which the top of the outlet is flush with the bottom of the vat, is a definite improvement on Type A, but still permits the pump to suck air before the vat is properly empty, especially with viscous cream. Type C, in which the outlet is from the bottom of the vat, has similar disadvantages to Type B. Type D, with a small well at the end of the vat, and the outlet connected with the bottom of the well, is the most satisfactory. It permits an almost complete draining of the vat before the pump can suck air, and an easy removal of residual froth by use of a small quantity of water.

## CALIBRATION OF THE NEUTRALIZING-VAT

The recommendation of Valentine(8) that the calibration of the vat should be carried out by weighing cream into the vat is in error. The measurement of cream for determination of acidity is by volume, and in converting the titration to a figure for percentage lactic acid, the assumption is made that the pipette delivers 9 g.—i.e., that the density of the cream is the same as the density of water. Cream actually is less dense than water, but the error of the assumption is cancelled out if the vats also are calibrated by volume. The calibration may be done by mathematical calculation, but with vats with a sloping bottom and a curve that may not be truly cylindrical, or if the vat changes in shape under load, the calculation is open to some error. The best method is by direct measurement of cold water into the vat, either by weighing, say, 100 lb. successively on the scales and tipping it into the vat, or by use of a 10-gallon measure. If the vat has deep, vertical sides and a curved bottom, the calibration need only be continued until the vertical section is reached, after which the calculation of volume from measurement is simple. If the measurement of quantity of cream is by

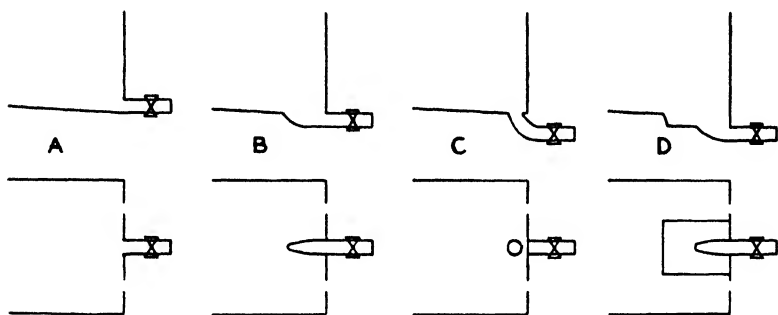


FIG. 1.—Elevation and plans of outlets for neutralizing-vats.

weight of cream added to the vat, the measurement of quantity of cream taken for the acidity test should also be by weight. An appreciable error due to the use of a pipette is not likely to occur except with high-acid creams containing large quantities of gas. Newman and Wiley(8) quote an instance where, owing to gassiness in the sample as pipetted, the weight of cream measured in a 9 ml. pipette was only 7.3 g.

## CHECKING OF NEUTRALIZATION BY TITRATION OF PASTEURIZED CREAM

The accuracy of neutralization is usually checked by an acidity determination on a sample of cream from the water cooler. The check is open to three sources of error:—

(1) If the neutralization is being carried to a very low acidity, it is not always completed during pasteurization and some carbon dioxide remains in the cream, causing the acidity to appear too high. This carbon dioxide is largely lost during churning, and the cream will then be overneutralized. The effect of the retained carbon dioxide on the acidity titre can be avoided if, before the titration, the sample is measured into a butter-moisture basin, boiled for a short time, and then cooled.

(2) If the cream is grossly overneutralized with sodium bicarbonate, the check test may show a higher acidity than if the correct amount had been added. Sodium bicarbonate is *slightly acid* to phenolphthalein at the pH of the normal end-point colour, and the presence of a large proportion of undecomposed bicarbonate will increase the apparent acidity. This defect also is shown up by boiling the sample before titration. If the cream turns pink when the phenolphthalein is added after boiling, it has been grossly overneutralized, and the degree of overneutralization can be determined by titration back to a slight pink colour with 0.1N-sulphuric acid.

(3) Cream treated in the vacreator becomes diluted to some extent by condensed water from the injected steam, and there is a reduction in the final acidity from this cause. A 10 per cent. dilution will reduce the final acidity of the cream by about 0.01 if the acidity aimed at is of the order of 0.08-0.10 per cent. lactic acid.

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## FIELD TRIALS WITH BORDEAUX MIXTURE FOR CONTROL OF RIPE-SPOT (*NEOFABRAEA* *MALICORTICIS*)

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### Summary

An account is given of the use in commercial orchards of summer Bordeaux sprays for the control of ripe-spot in Sturmer apples.

Field observations made in the Nelson Province and examination of fruit held in cool store show that the recommended programme of Bordeaux 2-6-100 in mid-January followed by Bordeaux 1-4-100 in mid-February—

- (a) Gives commercial control of ripe-spot in the orchard :
- (b) Produces marked improvement in ripe-spot control in cool store :
- (c) Does not cause observable foliage damage :
- (d) Leaves conspicuous, but not commercially objectionable, spray residues.

Evidence presented indicates that use of Bordeaux sprays may reduce the incidence of superficial scald in cool store, but tend to increase wilting of fruit.

FOLLOWING a series of experiments with Bordeaux mixture for control of ripe-spot of Sturmer apples, tentative recommendations were made for a commercial spray programme (Taylor, 1946). Several growers in the Nelson district made use of this or similar programmes, and in the present paper an account is given of the results secured as compared with those from the sulphur-spray treatments normally used. In addition to field observations, records are included from sample cases of fruit held in cool store. The influence of fruit maturity at time of picking on subsequent development of ripe-spot in cool store is also discussed.

### SPRAY TREATMENTS APPLIED

The recommended spray treatment consisted of Bordeaux 2-6-100 in mid-January, followed by 1-4-100 in mid-February with lime-casein (equal parts powdered casein and hydrated lime), 8 oz. to 100 gallons in each spray. Growers did not always adhere to this programme, and in such cases the actual programme is given in the text or tables.

Spray programmes normally employed vary from one grower to another. Individual treatments are not recorded, but in essentials consisted of lime-sulphur (15 per cent. polysulphides W W), 1 gallon to 150 gallons water plus colloidal sulphur (40 per cent. sulphur W W) 2 lb. to 100 gallons. In text and tables these treatments are referred to as "normal" spray treatments.

In both recommended and normal spray treatments acid lead arsenate was included at 1½ lb. to 100 gallons water with the addition of 3 lb. of hydrated lime in the normal spray programme.

### FIELD TRIAL IN SEASON 1942-43

Forty Sturmer trees in an orchard in the Stoke District, Nelson, were sprayed with the recommended Bordeaux programme and comparisons made with an equal number of trees receiving a normal sulphur treatment. Two applications of Bordeaux were made, the first on 21st January and the second on 26th February, normal sulphur sprays being applied to the adjacent block on each occasion.

Records of ripe-spot infection and fruit damage were taken by observation at harvest-time on 14th April and from the grading records of the packed crop. Sample cases of fruit were held in cool store.

### Field Records

On the Bordeaux treatment fruit developed a smooth bronze russet and a small amount of "copper injury" of the type described by Taylor(1946). The degree of damage was insufficient to affect the grade of fruit,† there being 260 cases of Fancy and 28 cases of Commercial grade fruit from the Bordeaux sprayed trees and 246 cases Fancy and 27 cases Commercial from the normal sulphur treatment.

Records were not taken of ripe-spot at the time of picking, but examination of reject fruit in the packing-shed showed that 0.7 per cent. of the total crop from Bordeaux treatment was severely infected and 10.8 per cent. from normal sulphur treatment. The rapid development of ripe-spot in the orchard at this period of the year was shown by two trees from each treatment which were left unpicked until the 20th April—i.e., seven days after the main harvest. From Bordeaux sprayed trees 3.7 per cent. of fruits were infected and from those receiving normal treatment 52.9 per cent.

### Cool Store Records

Cases of fruit from each treatment were held in cool store at Stoke, Port Nelson, and Christchurch until 2nd November. Results given in Table I show that the use of Bordeaux sprays gave marked improvement in control of ripe-spot.

TABLE I.—RIPE-SPOT ON COOL-STORED FRUIT FROM ORCHARD H. 224 RECEIVING BORDEAUX AND NORMAL SPRAY PROGRAMMES. (RECORDED 2ND TO 4TH NOVEMBER, 1943)

Cool Store.	Treatment.				Percentage of Fruit Infected with Ripe-spot *	
					Slight†.	Severe†.
Stoke .. ..	Bordeaux .. ..	..	..	..	12.7	0.6
	Normal sprays .. ..	..	..	..	33.3	2.2
Nelson .. ..	Bordeaux .. ..	..	..	..	14.9	Nil
	Normal sprays .. ..	..	..	..	33.5	3.2
Christchurch ..	Bordeaux .. ..	..	..	..	12.9	1.6
	Normal sprays .. ..	..	..	..	35.1	7.0
Mean percentages: Bordeaux sprays .. ..				..	13.5	0.7
Mean percentages: normal sprays .. ..				..	34.0	4.1

\* Percentages are based on examination of five cases of each treatment from Stoke and Nelson and six cases of each treatment from the Christchurch cool store.

† Included in "slight" grade are fruits with lesions of total area not greater than a circle of  $\frac{1}{4}$  in. diameter. In "severe" grade are all fruits with more extensive lesions.

‡ In commercial practice up to 75 per cent. smooth russet is allowed in the Fancy grade and over 75 per cent. in Commercial.

Records of "wilt" ‡ were taken in all three stores by grading on an arbitrary scale into slight, medium, and severe on the general appearance of each case of fruit. Of sixteen cases from Bordeaux treatment one showed slight, ten medium, and five severe "wilt," and from normal spray treatments ten slight, six medium, and none severe. The evidence strongly suggested that Bordeaux tended to increase degree of wilting.

It was noted that superficial scald was developing on some of the fruits, and here again an arbitrary scale of slight, medium, and severe was adopted for recording purposes. In the Bordeaux treatment one case showed slight, thirteen medium, and two severe. From normal spray treatment none were slight, three medium, and thirteen severe. Thus the use of Bordeaux sprays appeared to have reduced susceptibility of fruit to superficial scald.

TABLE II.—RIPE-SPOT INFECTION ON FRUIT HELD IN NELSON COOL STORE UNTIL 11TH OCTOBER, 1944

Locality of Orchard.*	Treatment.	Percentages Fruit Infected	
		Slight ‡.	Severe ‡
Waimea .. ..	Bordeaux 2-6-100 early February ..	15	0
	Normal sprays .. ..	34	1
Moutere Hills ..	Bordeaux 2-6-100 early February ..	5	0
	Normal sprays .. ..	22	3
" .. ..	Bordeaux as recommended .. ..	4	0
	Normal sprays .. ..	24	0
" .. ..	Bordeaux as recommended .. ..	5	0
	Normal sprays .. ..	23	5
" .. ..	Bordeaux 2-6-100 late February ..	16	2
	Normal sprays .. ..	48	6
" .. ..	Bordeaux 3-6-100 early February. 1½-6-100 early March	22	0
	Normal sprays .. ..	50	23
Mean percentages: Bordeaux treatments .. ..		11	0.3
Mean percentages: normal treatments .. ..		33.5	6.3

\* In each location sample cases from Bordeaux and normal spray treatments are from same orchard.

‡ Included in "slight" grade are fruits with one or more lesions of total area not greater than ½ in. diameter. In "severe" grade are all fruits with more extensive lesions.

#### OBSERVATIONS ON COMMERCIAL USE OF SUMMER BORDEAUX IN SEASON 1943-44

Twenty growers who had used summer Bordeaux sprays on Sturmer trees were visited in the Nelson Province just prior to harvest time. Wherever possible, comparisons of ripe-spot infection and of fruit and foliage injury were made with trees which had received normal spray treatments in the same or in adjacent orchards.

‡ "Wilt" refers to the condition of fruit resulting from loss of water.



*Field Records*

In no instance was there evidence of increased foliage damage following use of Bordeaux sprays.

"Copper injury" of the lenticel type of red pigmentation was found on some fruits from all Bordeaux sprayed trees. Damage was not extensive and did not affect the grade of fruit. Copper induced russet was present, but here again it was insufficient to reduce the grade. In general, however, fruit was duller in appearance than that from trees receiving a normal spray treatment.

TABLE III.—RIPE-SPOT INFECTION ON FRUIT HELD IN CHRISTCHURCH AND AUCKLAND COOL STORES

Locality of Orchard.*	Treatment.				Percentages Fruit infected.
<i>Christchurch Store, recorded 25th July, 1944</i>					
Moutere Hills ..	Bordeaux as recommended ..	..	..	..	5
" ..	" ..	..	..	..	8
" ..	" ..	..	..	..	5
" ..	Bordeaux 2-6-100, early February ..	..	..	..	9
Waimea ..	Bordeaux 2-6-100, late February ..	..	..	..	9
Mean percentage : Bordeaux treatments ..					7.2
Moutere Hills ..	Normal sprays ..	..	..	..	38
" ..	" ..	..	..	..	61
" ..	" ..	..	..	..	29
" ..	" ..	..	..	..	20
Waimea ..	" ..	..	..	..	31
Mean percentage : normal treatments ..					35.8
<i>Auckland Store, recorded 25th July, 1944</i>					
Moutere Hills ..	Bordeaux as recommended ..	..	..	..	8
" ..	" ..	..	..	..	17
Waimea ..	" ..	..	..	..	21
Moutere Hills ..	Bordeaux 2-6-100, early February ..	..	..	..	17
" ..	" ..	..	..	..	18
Mean percentage : Bordeaux treatments ..					16.2
Moutere Hills ..	Normal sprays ..	..	..	..	57
" ..	" ..	..	..	..	84
" ..	" ..	..	..	..	29
" ..	" ..	..	..	..	51
Waimea ..	" ..	..	..	..	90
Mean percentage : normal sprays ..					62.2

\* Sample cases from Bordeaux and normal spray treatments are from different orchards and cannot be compared individually.

Spray residues were heavy in one or two instances and development of fruit colour had been retarded.

In all the orchards visited there was definite evidence of improved ripe-spot control as compared with adjacent orchards receiving normal spray treatments.

*Cool Store Records*

Growers were asked to give all cases of Bordeaux sprayed fruit a distinctive mark so that it could be recognized later in cool store. With six growers, where direct comparisons between Bordeaux and normal-sprayed fruit were possible, arrangements were made to hold sample cases from each treatment in the Nelson cool store. Results of examination of the stored fruit given in Tables II and III show that marked improvement in ripe-spot control was secured by the use of Bordeaux sprays. In the Auckland store development of ripe-spot was more extensive than at Christchurch. At Nelson records were taken nearly two and a half months later than at Christchurch, but, in spite of this, ripe-spot infection was no greater. All three stores were held at a temperature of 36 to 37 c., and although small variations may have occurred, it seems unlikely that they would have been sufficient to cause such wide differences in ripe-spot development. A more likely explanation is that during transit to Auckland fruit advanced in maturity and consequently became more subject to development of ripe-spot. The importance of fruit maturity in this respect was discussed by Taylor and Brien(1943), and is referred to again in the next section.

## FRUIT MATURITY IN RELATION TO RIPE-SPOT DEVELOPMENT IN COOL STORE

A block of thirty-two Sturmer trees which had received a normal spray treatment was divided into four plots with eight trees in each. Portion of the fruit was picked on the 9th April and a sample case of at least 138 fruits from each plot was put into cool store: a second series of four cases was picked and placed in store on 21st April, and a third picking made on 5th May. An additional series of four cases was packed on 21st April, but held in a packing-shed until 5th May before placing in cool store. On each occasion fruit visibly free of ripe-spot was selected for the sample cases, and each apple was wrapped in paper before packing. Ripe-spot developed rapidly over the picking period, and by 5th May considerable difficulty was experienced in finding sufficient fruit free of ripe-spot for the sample cases. Consequently, there was a tendency to select greener fruit from the more shaded portion of the trees, where the incidence of ripe-spot is usually lower. As a result the sample cases were not as far advanced in maturity as they would normally be by this date.

The sample cases were held in cool store at Nelson until 18th November, when they were examined for the development of ripe-spot.

TABLE IV.—SHOWING EFFECT OF FRUIT MATURITY ON DEVELOPMENT OF RIPE-SPOT IN COOL STORE. (RECORDED 18TH NOVEMBER, 1943)

Treatment.	Date of Picking.			Date of placing in Cool Store.			Percentage Ripe-spot Infection.
1	..	9th April	.. ..	9th April	.. ..	..	8.4
2	..	21st April	.. ..	21st April	.. ..	..	44.7
3	..	21st April	.. ..	5th May	.. ..	..	70.6
4	..	5th May	.. ..	5th May	.. ..	..	65.6
Standard error			.. ..	.. ..	.. ..	..	5.31
Difference for significance at 5 per cent.			.. ..	.. ..	.. ..	..	17.0

Results given in Table IV show a progressive increase in the development of ripe-spot in cool storage from the first to the last picking. Where fruit was picked on 21st April and held fourteen days before placing in cool store (treatment 3) a significant increase in ripe-spot development occurred as compared with fruit picked on the same day and placed immediately in cool store (treatment 2). Thus it appears that, as a result of the occurrence of advanced maturity during the fourteen days prior to placing in cool store, fruit had become more subject to development of ripe-spot lesions. A similar comparison of treatment 3 with fruit picked on the 5th May (treatment 4) shows that increased development of ripe-spot in cool store did not occur in spite of the fact that fruit had remained on the trees for an additional fourteen days. It is apparent, therefore, that the increased development of lesions in cool storage on the later pickings was not due to higher incidence of incipient infection, but to advanced maturity of the fruit. Support is given to this view by evidence previously reported from spraying trials (Taylor, 1946), which indicates that the period of maximum ripe-spot infection occurs prior to the commencement of harvest.

#### CONCLUSION

From results secured in orchards in the Nelson Province it appears that the recommended treatment of Bordeaux 2-6-100 in mid-January and 1-4-100 in mid-February, or some modification of it, can be relied upon to give commercial control of ripe-spot. Foliage damage, if any, is very slight, and, although fruit damage does occur, it is of little or no commercial significance. Spray residues are sometimes heavy, but are not commercially objectionable.

On Bordeaux-treated fruit in cool store, development of ripe-spot is greatly retarded as compared with the development (of the organism) on fruit receiving normal spray treatments. Differences are most apparent where the interval between picking and placing in cool store is prolonged.

There is evidence to suggest that Bordeaux-sprayed fruit is likely to be more resistant to superficial scald, but more subject to "wilting" than fruit from the normal spray programmes. The commercial significance of these two factors has not been investigated.

Fruit maturity at time of picking appears to have considerable influence on development of ripe-spot in cool store. It seems probable that considerable improvement in control could be secured by harvesting at the earliest date practicable and placing fruit in cool store with a minimum of delay.

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## TRIALS OF NEW ZEALAND PASTURE SEEDS IN BRITAIN: 1941 AND 1942 TRIALS

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### Summary

Under New Zealand conditions of climate and farm management, highly productive strains of European grass and clover species have evolved. These have been further improved by breeding and their performance assured under a certification scheme. Much of this seed has been finding its way through the seed trade to grassland farming in other countries, particularly England. In 1941 and 1942 seed of the main New Zealand pasture strains was compared with British commercial and Aberystwyth Plant Breeding Station bred strains in a series of trials throughout England. Useful broad results were obtained from these trials; because of their nature it was not possible to obtain detailed results. In general, both the New Zealand and Aberystwyth strains were definitely superior to commercial strains. New Zealand certified perennial rye-grasses proved to have superior seedling establishment and vigour in the establishment year. In the following seasons it showed itself a good rye-grass and was very similar in many respects to the Aberystwyth S24 (pasture-hay type). New Zealand certified Italian rye-grasses also proved to have good establishment vigour and were slightly more persistent than other strains tried. New Zealand cocksfoot suffered from severe competition with the New Zealand rye-grasses and consequently did not show up as well in performance as Aberystwyth strains. In type they were somewhat similar to the Aberystwyth S191 (hay) strain. The New Zealand certified "mother" white clovers were so similar to the Aberystwyth S100 strain as to be indistinguishable in the field. Both are excellent clovers for dairy pastures. New Zealand certified Montgomery red clover was similar to English late flowering, but was not quite as persistent as the Aberystwyth S123 strain.

### INTRODUCTION

THE high-producing permanent pastures and the grass leys of New Zealand have sprung from traditional European agriculture, and the component species are European grasses and clovers. Under European systems of agriculture many different strains of these species have evolved; the red-clover strains in Britain alone are numerous. Further, the incursions of science into agriculture have seen the introduction of other strains having their origin in breeding.

It is therefore not surprising that, upon introduction into New Zealand, these European grass and clover pasture species have developed, by natural selection, strains peculiar to the new farming and climatic conditions. Thus a relatively persistent, semi-pasture type of cocksfoot (*Dactylis glomerata*)

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evolved on the Akaroa Peninsula, a semi-pasture, persistent type of rye-grass (*Lolium perenne*) evolved in Hawke's Bay and other places, and a vigorous, large-leaved type of white clover (*Trifolium repens*) appeared in certain parts of Canterbury. These types were first worked upon scientifically by Levy and Davies(1, 4, and 5) and the best of them served as the basis for breeding-work to produce further improvements. Seed production from the best of the so-called regional strains, and later the bred strains, was safeguarded and fostered by the introduction of the New Zealand Government certification scheme in 1930.

Recently selected Welsh strains of Montgomery red clover (*Trifolium pratense*) and Italian rye-grass (*Lolium multiflorum*) were introduced into New Zealand from Britain. After further selection and breeding, seed production of these strains has also been carried out under the certification scheme.

Seed production in the Dominion has, in general, reached a high standard. Constancy of type and analysis (purity and germination of the sample) is ensured under the certification scheme, and climatic conditions usually enable a good sample of the seed to be produced. This has led, over a period of years, to the building-up of a steadily expanding export trade, with Britain as the chief market(6).

At the Grassland Conference held in England in 1938, arrangements were made with the British Ministry of Agriculture to carry out a series of trials of New Zealand pasture seeds in Britain. The object was to verify the type and performance of the New Zealand strains under British conditions. These trials were not commenced until 1941, and in that year a mixture of all New Zealand seeds was included in a series of English grassland demonstration areas established under the auspices of the Grassland Improvement Station, Drayton, Stratford-on-Avon.

#### TYPE OF TRIAL

The trials form part of a series at first carried out under a grant made by the Royal Agricultural Society of England to the Welsh Plant Breeding Station, Aberystwyth. Subsequently, when the Grassland Improvement Station, Stratford-on-Avon(7), was set up in 1940, the work was financed chiefly by the Ministry of Agriculture(3).

The series was commenced in 1937, and up to 1944 consisted of a number of widely scattered centres at each of which from three to six 1-acre plots of comparable seed mixtures, but of different strains, were sown. It was hoped to throw light upon problems connected with the reseedling of extensive acreages of low-producing permanent pastures throughout England.

At this point it will be opportune to give a brief description of British grasslands at the commencement of the trials. An accurate account has been made available by a survey carried out by Davies *et al.*(2).

In 1939 in England and Wales there were 8,396,941 acres of arable land, including 1,827,114 acres of temporary leys. The remaining agricultural land consisted of 15,702,000 acres of permanent grass and 5,601,000 acres of rough hill grazings. The survey of Davies *et al.* showed these permanent grasslands to consist of the types shown in Table I.

TABLE I.—ACREAGES AND TYPES COMPRISING THE PERMANENT GRASS AND ROUGH GRAZINGS OF ENGLAND AND WALES (from the 1939 survey by Davies *et al.*)

Type	Area (Acres).	Percentage.
First-grade rye-grass pasture . . . . .	251,000	1.2
Second-grade rye-grass pasture . . . . .	912,000	4.4
Third-grade rye-grass pasture . . . . .	4,317,000	20.2
<i>Agrostis</i> pasture, including <i>Agrostis</i> with fescue topgrass, &c. . . . .	9,580,000	45.0
<i>Agrostis</i> , with rushes and sedges of rough grazings . . . . .	734,000	3.4
Categories of rough grazings, including Down and Cotswold fescue swards, <i>Nardus</i> and <i>Molinia</i> Moors, &c. . . . .	5,525,000	25.8
Total permanent pasture and rough grazings . . . . .	21,319,000	100.0

It was these large areas of low-producing permanent pasture and rough grazings which presented the greatest grassland problems. The trials, parts of which are dealt with in this report, were designed to establish a satisfactory technique for reseeding these lands ex-turf, or seeding down under a crop in rotation. At the same time they were to demonstrate the greater productivity of a well-established and properly managed ley sown down to good strains of the component species in normal use and in sensible seed mixtures.

The centres for the trials have been chosen in conjunction with the county agricultural staffs, and the laying-down and supervision of the sowing has frequently been their responsibility. Management of the established pasture has been a matter for the farmer to fit into his routine farm practice, though he has usually enlisted and taken the advice of the county and the Grassland Improvement Station. Fertilizing has also been a matter for the farmer to decide, and during the war he has depended largely upon the available wartime supplies of fertilizers. There has thus been, and advisedly so, no uniformity in the treatment and management of these trials.

The following more complete description of the trial centres refers to those at which New Zealand seeds were included in the seed mixtures in 1941 and 1942. The centres were scattered throughout England in a number of widely separated counties. They were laid down on the widest range of soil types varying from light loams to the heaviest clays. Included in the series was a heavy river silt, fen (a silty peat), light residual soil on chalk, clay with flints on chalk, and clays including boulder clays of glacial origin.

Because of the great variations of soil types associated with the English countryside it is difficult to give an estimate of the area of agricultural land that each trial represented. From a vegetation point of view, however, it can be said that they were situated on areas characteristic of the *Agrostis* and *Agrostis* with rye-grass pasture types, which account for some 45 per cent. (9,580,000 acres) of the 1939 area of permanent grassland in England and Wales(2). These pastures were mainly poor to derelict with an *Agrostis* or *Agrostis*-fescue base, sometimes associated with small amounts of white clover. In some areas (Fig. 1) tussock-grass (*Deschampsia caespitosa*) and tor-grass (*Brachypodium pinnatum*)—both weeds in a pasture—and other weed species formed important elements in the sward. These weed grasses indicated a stage of dereliction, for ant-hills, thorn and scrub, *Crataegus*, *Prunus*, *Rubus*, *Rosa*, &c., soon followed (Fig. 2).



FIG. 1.—Derelict English grassland on a heavy clay. Tor-grass (*Brachypodium pinnatum*), a weed species, together with *Agrostis* spp., are the dominant grasses. Numerous ant-hills are scattered about.



FIG. 2.—Derelict English grassland being invaded by bramble and thorn (*Rubus* and *Crataegus* spp.).

Most of the centres were situated in the rainfall belt below 30 in., so that the necessity for conserving soil moisture during establishment was an essential problem.

In 1941, thirty trials were sown, each consisting of five adjacent 1-acre plots sown with a somewhat complicated seed mixture. From a comparative point of view, only three of the plots were of direct interest to New Zealand. The details of the mixtures in these are shown in Table II.

TABLE II.—SCHEDULE OF SEED MIXTURE IN 1941 R.A.S.E. TRIALS

Species and Strain.	1. Including Aberystwyth Strains (Pounds per Acre).	2: All Commercial Strains (Pounds per Acre).	3: Chiefly New Zealand Strains (Pounds per acre)
<i>Italian Rye-grass</i> —			
Commercial .. .. .	6	6	..
New Zealand certified mother .. ..	..	..	6
<i>Perennial Rye-grass</i> —			
Commercial .. .. .	6	16	..
Aberystwyth S23 (pasture), S24 and S101 (pasture hay)	10	..	..
New Zealand certified mother .. ..	..	..	16
<i>Cocksfoot</i> —			
Danish type .. .. .	2	8	..
Aberystwyth S191 (hay), S143 and S190 (pasture)	6	..	..
New Zealand certified "Akaroa" .. ..	..	..	8
<i>Timothy</i> —			
Commercial .. .. .	1	4	4
Aberystwyth S51 (pasture), S48 (hay), and S50 (pasture)	3	..	..
<i>Red Clover</i> —			
English late flowering .. .. .	..	4	..
Aberystwyth S123 .. .. .	4	..	..
New Zealand certified Montgomery .. ..	..	..	4
<i>White Clover</i> —			
Kentish .. .. .	$\frac{1}{2}$	1	..
Aberystwyth S100 .. .. .	$\frac{1}{2}$	..	..
New Zealand certified mother .. ..	..	..	1
<i>Trefoil</i> —			
Commercial .. .. .	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
<i>Alsike</i> —			
Canadian .. .. .	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
Pound of seed per acre .. ..	40	40	40

## SUMMARY

- Mixture 1 : Chiefly Aberystwyth strains with inclusion of some commercial strains.  
 Mixture 2 : All commercial strains.  
 Mixture 3 : Chiefly New Zealand strains.

It is thus seen that the comparison was mainly one of mixtures containing Aberystwyth, commercial and New Zealand strains.

In the 1942 series of trials, twenty-nine centres included New Zealand seed mixtures. This series was designed along the same lines as the 1941 series, but the mixtures were simpler and the comparisons more direct.



Ten of the centres included a mixture of certified New Zealand "pedigree" seed as well as certified New Zealand "mother" seed. The schedule of mixtures used in these trials is given in Table III.

TABLE III.—SCHEDULE OF SEED MIXTURES IN 1942 R.A.S.E. TRIALS

Species and Strain.	1.	2.	3.	4.	5.
<i>Italian Rye-grass</i> —					
Irish .. .. .	6	6	6	..	..
New Zealand certified mother .. .. .	..	..	..	6	..
New Zealand certified pedigree seed .. .. .	..	..	..	..	6
<i>Perennial Rye-grass</i> —					
Irish .. .. .	16	..	..	..	..
Aberystwyth S24 .. .. .	..	16	..	..	..
Aberystwyth S23 .. .. .	..	..	16	..	..
New Zealand certified mother .. .. .	..	..	..	16	..
New Zealand certified pedigree seed .. .. .	..	..	..	..	16
<i>Cocksfoot</i> —					
American .. .. .	6	..	..	..	..
Aberystwyth S37 .. .. .	..	6	..	..	..
Aberystwyth S143 .. .. .	..	..	6	..	..
New Zealand certified mother .. .. .	..	..	..	6	..
New Zealand certified pedigree seed C23 .. .. .	..	..	..	..	6
<i>Red Clover</i> —					
Early broad .. .. .	4	..	..	..	..
American Mammoth .. .. .	..	4	..	..	..
Aberystwyth S123 .. .. .	..	..	4	..	..
New Zealand certified Montgomery mother .. .. .	..	..	..	4	..
New Zealand certified Montgomery pedigree .. .. .	..	..	..	..	4
<i>White Clover</i> —					
New Zealand uncertified .. .. .	1	..	..	..	..
Aberystwyth S100 .. .. .	..	1	..	..	..
Kentish type .. .. .	..	..	1	..	..
New Zealand certified mother .. .. .	..	..	..	1	..
New Zealand certified pedigree seed .. .. .	..	..	..	..	1
Trefoil .. .. .	1	1	1	1	1
Alsike .. .. .	2	2	2	2	2
Pound of seed per acre .. .. .	36	36	36	36	36

## SUMMARY

- Mixture 1 : Chiefly commercial strains.  
 Mixture 2 : Chiefly Aberystwyth leafy hay strains.  
 Mixture 3 : Chiefly Aberystwyth pasture strains.  
 Mixture 4 : New Zealand certified mother strains.  
 Mixture 5 : New Zealand certified pedigree strains.

The general nature, lack of uniformity in management, and relatively complicated seed mixtures of both the 1941 and 1942 series have made it possible only to obtain information of a broad nature from them. While a number of selected trials have been observed consistently over a period of four to five seasons, no detailed records or statistical analyses have been possible. Nor has it been possible to obtain comparative production figures. On the other hand, it has been possible to collect much useful information on strains, persistency, &c. This information has been strengthened by inspection of a number of observation plots sown at such centres as the National Institute of Agricultural Botany, Cambridge, North of Scotland Agricultural College, county farm institutes, &c.

The problem of successful establishment, which was one of the most important to English investigators, has been largely solved by the complete series of trials. This has been of little importance from a New Zealand point

TABLE IV.—PERCENTAGE COVER OF HERBAGE SPECIES IN 1941-SOWN (ENTRIES (ARRANGED BY SEASON)

Species.	Autumn, 1941, Plots.						Spring, 1942, Plots.			Autumn, 1942, Plots.			Spring 1943, Plots.			Autumn, 1943, Plots.			Spring, 1944, Plots.			Autumn, 1944, Plots.		
	1			2			1			1			1			1			1			1		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Perennial rye-grass	46	47	48	47	44	47	45	37	50	42	35	51	42	41	48	46	40	55	48	41	48	46	40	55
Italian rye-grass	2	2	5	3	2	5	6	7	5	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1
Cocksfoot	1	1	1	5	8	1	4	4	2	2	8	5	8	8	7	7	6	6	7	8	7	7	6	6
Timothy	1	1	1	2	1	2	3	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Other grasses	3	1	1	5	4	3	3	6	4	4	7	3	3	3	5	4	9	2	3	3	5	16	24	12
Red clover	8	8	7	4	6	6	7	5	5	4	4	5	4	7	11	7	12	8	2	17	11	7	12	8
White clover	4	3	4	5	4	5	9	16	10	17	21	12	11	11	11	11	11	11	11	11	11	11	11	11
Alsike and Trefoil	1	1	1	2	2	2	1	6	2	4	4	3	1	1	1	1	1	1	1	1	1	1	1	1
Weeds	3	3	3	2	2	2	5	6	2	4	4	3	4	6	4	2	3	3	4	6	4	2	3	3
Bare ground	32	33	31	25	27	25	20	21	20	16	17	16	16	17	18	13	13	13	16	17	18	13	13	13
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Summary to table—																								
Total sown grasses	49	52	54	57	55	58	55	46	58	54	46	60	53	53	60	62	48	64	53	53	60	62	48	64
Total sown clovers	13	11	11	11	12	11	17	21	16	22	26	18	13	17	13	7	12	8	13	17	13	7	12	8
Total unsown plants	6	4	4	7	6	6	12	6	8	11	6	6	11	13	9	18	27	15	12	13	9	18	27	15
Total bare ground	32	33	31	25	27	25	20	21	20	16	17	16	16	17	18	13	13	13	16	17	18	13	13	13
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Plot No. 1. Chiefly Aberystwyth strains. Plot No. 2 Chiefly commercial strains. Plot No. 3 Chiefly New Zealand strains.

TABLE V.—PERCENTAGE COVER OF HERBACE SPECIES IN 1941-SOWN CENTRES (ARRANGED BY PLOTS)

Species.	Plot 1.						Plot 2.						Plot 3.					
	Autumn, 1941.	Spring, 1942.	Autumn, 1942.	Spring, 1943.	Autumn, 1943.	Spring, 1944.	Autumn, 1944.	Autumn, 1941.	Spring, 1942.	Autumn, 1942.	Spring, 1943.	Autumn, 1943.	Spring, 1944.	Autumn, 1944.	Autumn, 1941.	Spring, 1942.	Autumn, 1942.	Spring, 1943.
Perennial rye-grass	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Italian rye-grass	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cocksfoot	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Timothy	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Other grasses (unsown)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Red clover	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
White clover	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Alsike and Trefoil	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Weeds	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Bare ground	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Total ..	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Summary to table—	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Total sown grasses	49	57	55	54	..	59	62	52	46	46	46	..	53	48	53	58	58	60
Total sown clovers	13	11	17	22	..	13	7	11	21	21	26	..	17	12	11	11	16	18
Total unsown plants	6	7	8	8	..	12	18	4	6	12	11	..	13	27	4	6	6	6
Total bare ground	32	25	20	16	..	16	13	33	27	21	17	..	17	13	31	25	20	16
Total ..	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Plot No. 1: Chiefly Aberystwyth strains. Plot No. 2: Chiefly commercial strains. Plot 3: Chiefly New Zealand strains.

of view, because, at each centre, the New Zealand seeds shared the same fate—establishment or failure—as the accompanying Aberystwyth or commercial seeds. In other words, the whole centre either established or failed to do so according to weather and other factors.

Features which did show themselves in the trials which became established were relative persistency of strains, earliness of growth, type of pasture formed, susceptibility of rye-grasses to rust (*Puccinia graminis* vars.), and palatability.

With regard to relative persistency of strains, an attempt was made to measure this by making a pasture cover analysis of selected trials in the spring and autumn of each year. This was done by estimating by eye the percentage cover of grasses and clovers, at some 2 in. from ground-level, in ten random 6 in. square quadrats. There are, of course, many objections to the use of such a method. Seasonal growth, types of management, and method of fertilizing all alter radically the percentage of pasture species present at a particular time. Under the circumstances, however, it was the only method, commensurate with any degree of accuracy, which could be used. It must be emphasized, therefore, that the results can only be indicative and must be regarded in their broad implications.

Of the 1941 centres, a number were observed and percentage cover analysis taken periodically. For some ten of the trials this was done regularly in the autumn and winter up to the autumn of 1944. Tables IV and V give the results of the analyses for these ten centres.

The following comments based upon these analyses and also analyses and inspection of a number of other centres can be made:—

*1941 Series of Trials* (Figs. 3 and 4).—Three plots in each of these trials were studied: plot 1, mainly Aberystwyth strains: plot 2, mainly commercial strains: plot 3, mainly New Zealand strains.

*1941 (Establishment Year)*.—Bearing in mind what has been said relative to the general question of establishment, the three plots in the first year showed minor, but significant, differences. In the New Zealand mixture the certified Italian rye-grass was better tillered and more vigorous than the "Commercial" (Irish Italian) product in the other two mixtures. New Zealand certified "mother" perennial rye-grass also showed greater seedling vigour than the other strains, though this was to be seen mainly in the appearance of the swards rather than as differences in the percentage cover.

The establishment and behaviour of the strains of cocksfoot and timothy did not have significant differences in the type of sward established in the seeding year. This was also largely true of the red and white clovers, though the S100 white clover in plot 1 and New Zealand certified mother white clover in plot 3 were larger leaved than the Kentish in plot 2.

It was the vigour of the New Zealand certified Italian rye-grass and seedling vigour of the New Zealand certified perennial rye-grass which produced the differences and gave the New Zealand plot a slight advantage in the establishment year.

*1942 Season*.—At the beginning of this season the New Zealand certified Italian rye-grass was still vigorous. This, together with the vigour of the New Zealand certified perennial rye-grass, which vigour appeared to increase by comparison with the other plots as the season advanced, had an adverse effect upon the cocksfoot and timothy content of the sward. These two species did not establish as well in the New Zealand plot as in the other two.

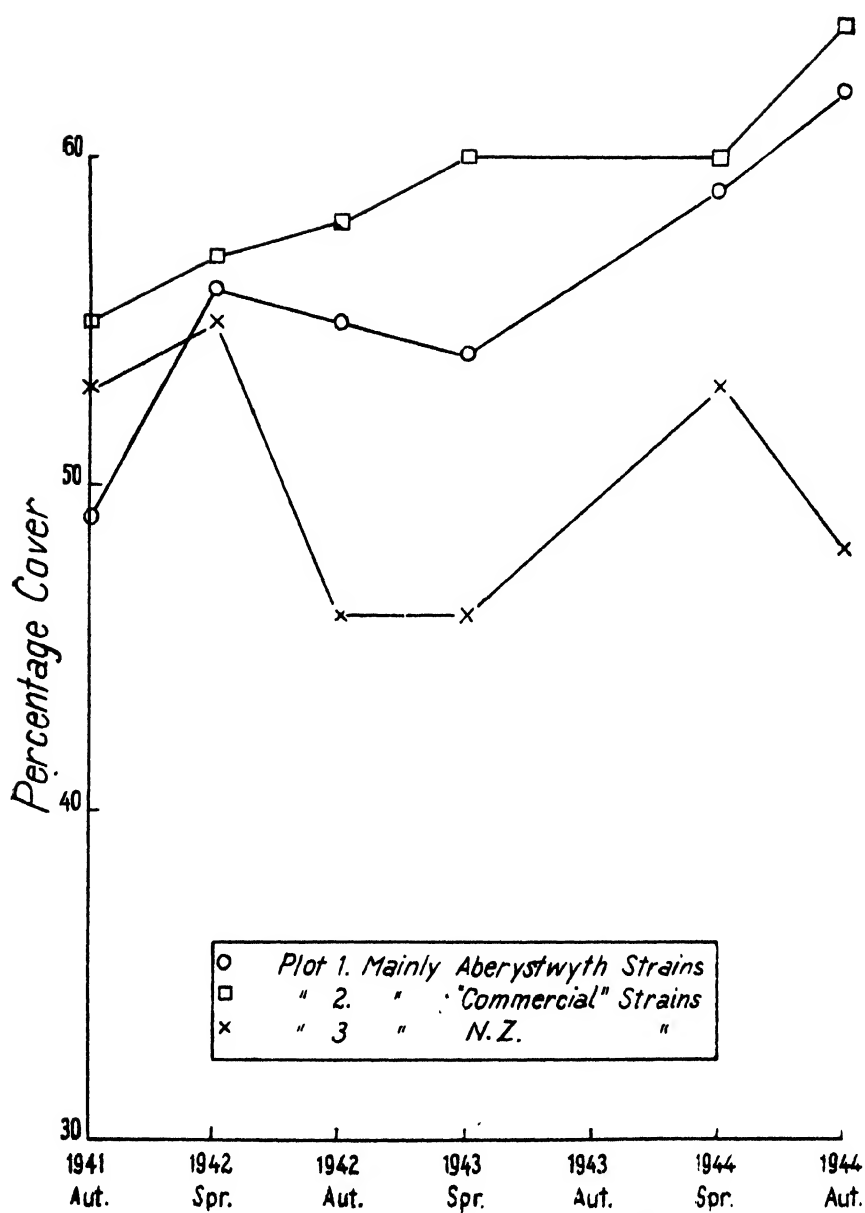


FIG. 3.—Graph of the fluctuation in percentage cover of total sown grasses in the 1941 trials.

By the end of the season Italian rye-grass was reduced to a small percentage in all three plots, but the comparative increase in perennial rye-grass was greatest in the New Zealand plot, which now became dominated by perennial rye-grass.

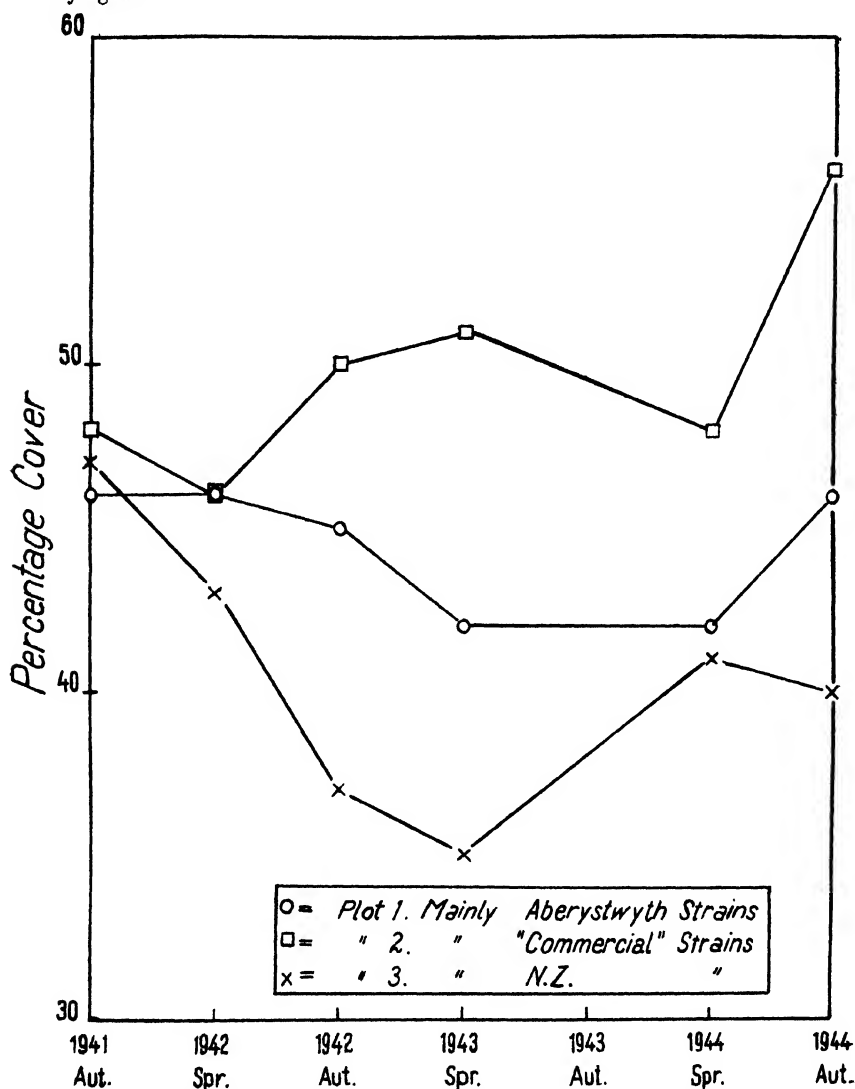


FIG. 4.—Graph of the fluctuation in percentage cover of perennial rye-grass in the 1941 trials.

By the autumn the amount of cocksfoot in the New Zealand plot was less than in the other two plots, this being a direct reflection on the competition with rye-grass rather than of strain difference as such. Where centres were out for hay in this year, the commercial cocksfoot (plot 2) bulked well, although it gave a more stemmy and therefore less leafy hay than the mixed Aberystwyth cocksfoot strains in plot 1 and the New Zealand rye-grass in plot 3.

The Aberystwyth strains of timothy increased throughout the season, and by the autumn contributed markedly more to the sward than the "commercial" timothy in plots 2 and 3.

All three strains of late-flowering red clover—Aberystwyth S123, English late flowering, and New Zealand certified Montgomery—apart from seasonal differences of growth, flowering, and tillering, gave much the same performance, though by the end of the season Aberystwyth S123 was slightly the better. The white-clover cover, irrespective of strain, fluctuated in competition with the grasses.

*1943 Season.*—By the spring of 1943 (second harvest year) the New Zealand certified rye-grass had increased by comparison with the rye-grasses in plots 1 and 2. Of the small amount of Italian rye-grass remaining in the three plots, New Zealand was the most abundant. The increase of perennial rye-grass in the New Zealand plot still took place at the expense of cocksfoot, timothy, and white clover. A pronounced decrease of the "commercial" perennial rye-grass in plot 2 was noticeable, and this thinning allowed the white clover to increase. The inclusion of mainly Aberystwyth strains in plot 1 prevented any thinning out of the rye-grass in that plot.

Sufficient analyses of pasture cover were not made in the autumn to obtain average figures. From trials inspected, however, it was obvious that cocksfoot and timothy were on the increase in all plots and approximately in the same proportion as in the previous season—i.e., the Aberystwyth strains were superior to the others.

*1944 Season.*—The New Zealand perennial rye-grass increased still further at the end of this season. The Aberystwyth strains in plot 1 also increased, while the "commercial" strain in plot 2 improved slightly over the previous season. This improvement was the result of shedding of seed. By the autumn the Italian rye-grass had largely disappeared, except for a small amount of the New Zealand strain.

Cocksfoot fluctuated throughout the season, with the advantage in favour of the Aberystwyth strains, though with New Zealand certified holding very well in competition with a strong rye-grass sward.

At the beginning of the season there was still some Aberystwyth S123 and New Zealand certified Montgomery red clovers, while English late flowering had virtually disappeared. By the end of the season the first two strains had also largely gone.

The autumn saw a very marked increase in unsown grasses, mainly *Poa trivialis*, especially in plot 2. This deterioration of the sward in all plots continued into the 1945 season. It could be attributed mainly to the lack of proper top-dressing with fertilizers consequent upon wartime restrictions.

*Trials sown in 1942.*—Previous mention has been made that in this series, consisting of twenty-nine centres each with five 1-acre plots, the seed mixtures were simpler and comparison of the different strains of the component species more direct than in the 1941 trials. Only one of these centres was followed in detail, though a number of others were visited from time to time. Brief mention only will therefore be made of this series.

From the schedule of seed mixtures in Table II it will be seen that plot 1 consisted of "commercial" strains, plot 2 of leafy hay Aberystwyth strains, plot 3 of dense-pasture Aberystwyth strains, plot 4 of New Zealand certified mother strains, and plot 5 of New Zealand certified pedigree strains.

As in the 1941 trials, the seedling vigour of the New Zealand certified Italian and perennial rye-grasses was marked. Both the certified mother and pedigree seed performed equally well and, except in a few trials, little difference could be seen between the two. This may be attributable to low status of soil nutrients and therefore insufficient fertility to bring out the best potentialities of the pedigree strains.

In the years following establishment the tillering capacity of S23 (leafy pasture type) showed itself, and this strain formed by far the densest, though by eye judgment not necessarily the most vigorously growing, sward. The plot containing commercial perennial rye-grass "opened out" quickly and allowed the incoming of unsown grasses. The plots containing S24 (leafy hay type) and New Zealand certified mother and pedigree showed up very similarly the one to the other.



FIG. 5.—Right: S23 perennial rye-grass, a dense pasture type. Centre: New Zealand uncertified perennial rye-grass, a mixed, lax, pasture-hay type. Left: New Zealand certified "mother" perennial rye-grass, a good pasture-hay type.

#### BEHAVIOUR OF THE INDIVIDUAL PASTURE COMPONENTS

*Perennial Rye-grass* (Figs. 5, 6, and 7).—The New Zealand certified strains have shown greater seedling and establishment vigour in the seedling year than either the commercial or Aberystwyth S23 strains. In the years following establishment they have shown about the same competitive ability, tillering capacity, &c., as S24 (leafy hay strain). On fertile soils, New Zealand pedigree strains have been better. For a period of years both S24 and the New Zealand strains can maintain or even slightly increase their percentage cover. By comparison, S23 (pasture type) shows greater tillering capacity and is able to form a very dense sward, while commercial strains are the least well tillered and show lack of persistency. Even in the first year after establishment, swards of the latter begin to open out and the white clover or unsown grass and weed contents increase.





FIG. 6.—Right : S23 perennial rye-grass, a dense pasture type. Left : New Zealand uncertified perennial rye-grass, a mixed, lax, pasture-hay type.



FIG. 7.—S23 perennial rye-grass being grown for seed production. This is a good dense pasture type of rye-grass.

The New Zealand, S24, and commercial strains all commence growth about the same time at the beginning of the season and are definitely earlier in growth than S23. In the autumn, New Zealand, S23, and S24 all show better growth than commercial strains, while S23 is the most winter green, and a dense pasture of this strain stands up to winter treading better than all other strains under test.

Palatability of the New Zealand and commercial strains is about the same as S24 and is in the spring greater than S23. In the summer, autumn, and winter the difference is not so marked. Palatability is a difficult point to assess, however, and alters at different times of the season.

The New Zealand certified strains appear to be the least susceptible of the rye-grasses to incidence of yellow rust (*Puccinia loliacea*). S23 is somewhat similar to the New Zealand strains in this respect, but varies from season to season. Commercial strains are the most susceptible.

*Italian Rye-grass*.—In the series of trials the only comparison of Italian rye-grasses has been between New Zealand certified and Irish commercial strains. New Zealand has shown up to advantage in all respects compared with the commercial. It has shown greater vigour of establishment, growth, and persistency, though the main superiority lies in greater tillering capacity and leafiness.

*Cocksfoot*.—In the second year of the 1941 trials all strains of cocksfoot established satisfactorily, though the New Zealand certified strain suffered slightly by greater competition of the New Zealand rye-grasses. The commercial strain (Danish type) produced the least well tillered plants, though the tillers were larger and broader than in any other strain. New Zealand certified strains showed better tillering than commercial and were somewhat similar to S191 (hay strain). The pasture strains, S190 and S143, produced the best tillered plants of all and individual plants were very dense.

Up to 1945 the trials did not indicate any difference in persistency in the strains.

Both the commercial and New Zealand strains start into growth slightly earlier than the Aberystwyth strains.

*Timothy*.—No New Zealand timothy strains were included in the trials, so the performance of this grass will not be dealt with in detail. It is worthy of note, however, that a constant and remarkable increase of the Aberystwyth timothy strains took place in some 1941 trials until in 1945 individual plots contained 20 to 25 per cent. of timothy cover.

*Red Clover*.—The late-flowering types—S123, English late flowering, and New Zealand certified Montgomery—have been compared. All established well in the 1941 trials and showed about equal performance, though S123 was somewhat the more persistent. By the end of the 1944 season (third harvest year) all strains had disappeared. There were differences in time of flowering and form of plants.

*White Clover*.—The white-clover content of the pastures, as would be expected, fluctuated with the grass component of the swards. It was therefore not possible to make a direct comparison of the strains used—namely, New Zealand certified mother and pedigree, S100, and Kentish. Other trial areas have, however, shown that New Zealand certified mother and S100 are very similar clovers and are now frequently accepted as interchangeable in pasture mixtures. Both are large leaved and vigorous by comparison with Kentish, which is smaller growing, though under adverse conditions more persistent. Kentish is essentially a sheep-pasture clover as compared with the other two, which can be regarded as white clovers particularly useful for inclusion in mixtures designed for cattle pasturage.

## ACKNOWLEDGMENTS

Grateful acknowledgment is made to Sir George Stapledon and the various members of the Grassland Improvement Station, Stratford-on-Avon, for making these trials possible and giving every assistance in inspections. The records of the station have always been accessible, and many inspections were made in collaboration with the Grassland Improvement Station's staff. Thanks are also extended to the many county agricultural officers for their active interest in the trials, and finally to Messrs. Wright, Stephenson, and Co., Ltd., for the generous supply of seed.

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# THE NEW ZEALAND JOURNAL OF SCIENCE AND TECHNOLOGY

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## LIME-INDUCED BORON DEFICIENCY IN TOBACCO AT UMUKURI, NELSON, NEW ZEALAND

By H. O. ASKEW, Cawthron Institute, Nelson

*Received for publication, 12th June, 1946*

### Summary

A stunting of tobacco plants accompanied by chlorosis and curling of the leaves which occurred on a coarse sandy soil in the second season after treatment with ground limestone has been diagnosed as being due to boron deficiency induced by the liming treatment.

Analyses of samples of soil and of the plants are given showing reduced boron contents where lime was used.

A warning is issued against too free use of limestone on sandy tobacco soils.

### INTRODUCTION

In January, 1941, an unusual condition of tobacco plants growing on certain plots at the Tobacco Research Station, Umukuri, Nelson, was reported to the author by Mr. L. G. Copp, of the Station staff. These plants were inspected in the field and samples of leaves and of soils were obtained for chemical analysis. This paper reports the results of the examination of this material.

### EXPERIMENTAL

The area of coarse sand from which the samples were drawn was used for a test of the effect of varying rates of application of ground limestone, three rates— $\frac{1}{2}$  ton, 1 ton, and 2 tons per acre—being used. This limestone was applied broadcast about mid-October in 1939. In addition, other plots received no limestone and others borax at the rate of 20 lb. per acre, the latter substance being mixed with the fertilizer applied in the rows. Half the fertilizer was applied one week before planting and the other half about three weeks after planting. A complete 3-8-6 (N, P, K.) fertilizer at 800 lb. per acre was used. The plants were set out in mid-November. In the 1939-40 season apparently nothing unusual developed in the crop on the various plots.

In the 1940-41 season a 3-8-8 fertilizer at 1,000 lb. per acre was used, 400 lb. being put in the rows before planting and 600 lb. as a side-dressing three weeks after planting. No limestone or borax dressings were given in this season. Plants were put out on 18-19th November, 1940. Rainfall in November and December was 3.57 in. and 2.46 in. respectively.

The plants apparently grew well until unusual symptoms appeared suddenly about 20th January, 1941, following heavy rain a short time previously. Notes on condition of the plants taken on 31st January were as follows:—

Control (no lime or borax): Apparently healthy, about 3 ft. to 4 ft. high; a small amount of mosaic present (see Fig. 1).

20 lb. borax: Plants apparently normal, and 3 ft. to 3 ft. 6 in. high (see Fig. 2).

1 ton ground limestone: Plants were stunted and only about 12 in. high. Leaves were very wrinkled and cupped downwards, young growing centre had died, and secondary growth had appeared. Some interveinal chlorosis was also present (see Fig. 3).

2 tons ground limestone: Plants were very stunted, being only 9 in. to 12 in. high. The symptoms were similar to those for the 1-ton treatment, but were more severe (Fig. 4).

No marked abnormalities appeared in the plants grown on the  $\frac{1}{2}$  ton treatment, hence no samples were obtained from these plots.

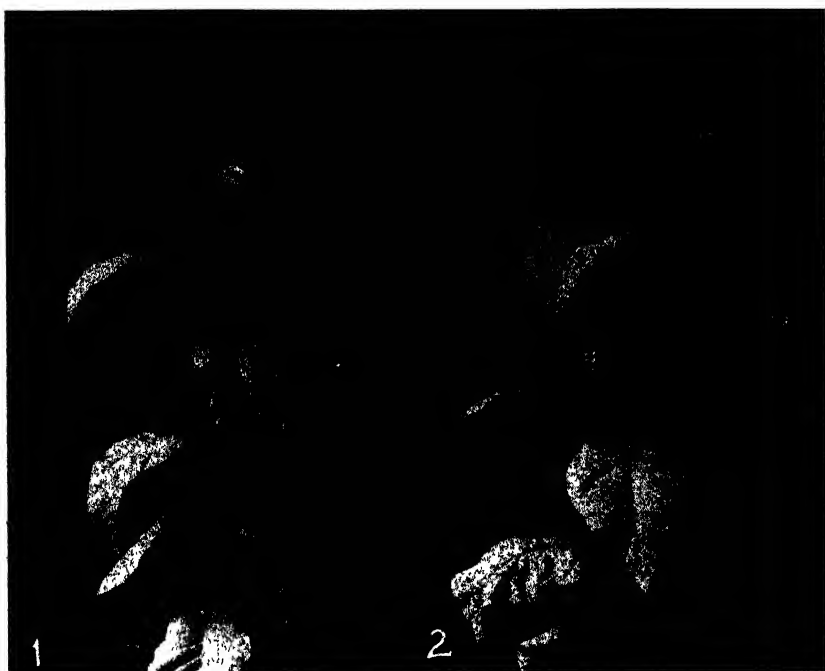
Soil samples were obtained from the above areas on 20th February, 1941, and analysed for base exchange capacity, exchangeable bases, and pH value. The exchangeable bases were extracted by 1N-ammonium acetate solution at pH 7.0. Calcium was precipitated as oxalate and determined by permanganate titration, magnesium was determined by the volumetric 8-hydroxy-quinoline method, and potassium by the volumetric cobaltinitrite method. pH values were determined in 1:2.5 soil and water suspensions by a quinhydrone electrode.

TABLE—I. EXCHANGEABLE BASES, AS MILLIGRAM-EQUIVALENTS PER 100 G. OF DRY SOIL, AND pH VALUES OF UMUKURI SOILS

Treatment.	Depth of Sampling (Inches).	Base Exchange Capacity (mg.-e.).	Total Exchangeable Bases (mg.-e.).	Calcium (mg.-e.).	Magnesium (mg.-e.).	Potassium (mg.-e.).	pH Value.
Control (no lime or borax)	0-3	6.96	3.84	3.59	0.41	0.41	6.25
	3-6	6.93	3.94	3.38	0.47	0.23	6.20
	6-12	5.91	3.62	3.03	0.51	0.18	6.20
Borax, 20 lb. per acre	0-3	..	3.19	2.90	0.42	0.40	6.45
	3-6	..	3.36	2.98	0.39	0.18	6.35
	6-12	..	2.65	2.30	0.37	0.16	6.45
Ground limestone, 1 ton per acre	0-3	..	4.18	3.43	0.30	0.46	7.10
	3-6	..	4.34	4.15	0.29	0.39	7.30
	6-12	..	2.86	2.56	0.29	0.37	6.95
Ground limestone, 2 tons per acre	0-3	..	5.14	4.36	0.31	0.46	7.35
	3-6	..	6.26	5.88	0.27	0.35	7.55
	6-12	..	3.74	3.35	0.26	0.30	7.20

The data in Table I show that on the control area, according to depth, the soil was 55-61 per cent. saturated with bases. These soil data also show that the borax treatment exerted no significant effect on the pH value, but that the limestone dressings raised the pH values to an extent sufficient to bring them into the range where minor elements such as borax are rendered less available to plants. The exchangeable calcium figure was greatly increased by the limestone, especially in the case of the 2-ton application. There was an indication that exchangeable magnesium had been depressed by the limestone.

Determinations of apparent residual carbonate (as  $\text{CaCO}_3$ ), by the vacuum volumetric method, in the two upper samples from the heavily



FIGS. 1 and 2.

Fig. 1. - Normal plant from control (no lime or borax) plot.

Fig. 2. - Apparently normal plant from plot treated with 20 lb. borax per acre in the previous season.



FIGS. 3 and 4.

Fig. 3.—Affected plant from plot receiving 1 ton of ground limestone per acre in the previous season.

Fig. 4.—Affected plant from plot receiving 2 tons of ground limestone per acre in the previous season.

limed area gave 0.050 per cent. and 0.063 per cent. respectively; the 0 in.-3 in. sample from the control area gave an apparent content of 0.019 per cent. These figures would indicate that only about 655 lb. of ground limestone per acre remained in the carbonate form in the top 6 in. of soil. It must be remembered, however, that the ground had been ploughed and cultivated after the applications had been made in the 1939-40 season, so that the limestone would be well distributed through the soil and some may even have been carried down to lower levels by rain; this was confirmed by the increased pH value and the small increase in the exchangeable calcium figure found at the deepest level of sampling.

Determinations of water-soluble boron in the soil from these plots, using the procedure of Berger and Truog(1) for the extraction and Maunsell's quinalizarin method(2) for determination of the boron, showed that the control area contained 0.2 p.p.m. of boron at each depth of sampling. This was reduced to approximately 0.1 p.p.m. boron on the limed areas. The previous borax application did not significantly increase the amount of water-soluble boron in the soil at the time of sampling. The coarseness of the soil has probably allowed efficient leaching of the applied borax to occur.

Analysis of whole-leaf samples taken from the various plots showed that there were some differences in the amounts of minerals present. The analytical data are given in Table II.

TABLE II.—MINERAL CONTENT OF TOBACCO LEAVES FROM LIMING TRIAL  
(Expressed as percentage of soluble ash, lime, magnesia, and potash and as p.p.m. of boron in the dry matter)

Treatment.	Soluble Ash (per Cent.).	Lime (per Cent.).	Magnesia (per Cent.).	Potash (per Cent.).	Boron (p.p.m.).
Control (no lime or borax)	11.17	3.22	0.48	2.86	6.3
Borax, 20 lb. per acre	7.94	1.93	0.25	2.45	26.7
Ground limestone, 1 ton per acre	10.02	2.79	0.29	2.63	3.9
Ground limestone, 2 tons per acre	11.14	3.47	0.36	2.48	3.5

These data show that for the main constituents sought for there are decreases in the soluble ash, magnesia, and potash contents following the use of lime. On the other hand, the use of borax has apparently had a still greater effect in reducing the amounts of these constituents in the dry matter of the leaves. It would appear, therefore, that the symptoms shown by the plants on the limed areas cannot be correlated with the variations in these constituents.

Although no important change in the soluble boron content of the soil on the borax-treated area was found, there was a great increase in the boron content of the leaves grown on it. Thus the borax-treated area gave 26.7 p.p.m. of boron and the control sample only 6.3 p.p.m. The roots of the plants may therefore have had access to boron in forms other than the water-soluble form, or may have penetrated to levels into which the applied borax had been carried by percolating rain. On the other hand, the boron contents of the leaves from the limed areas were not much more than half that of the control, being 3.9 p.p.m. and 3.5 p.p.m. for the 1-ton and 2-ton applications respectively. These low boron contents, together with the symptoms displayed by the plants, suggest that the unusual condition of the plants was brought about by reason of the boron in the soil being rendered less available to the plants following the use of heavy applications of ground limestone.

## DISCUSSION

Boron deficiency has been reported in solution or sand cultures of tobacco by a number of authors, but references to its occurrences in the field are not so frequent. It is moreover known that the use of lime as carbonate or oxide may induce boron deficiency in crops grown subsequent to such treatment. Why boron deficiency should appear is not precisely known; it can only be assumed that boron has been immobilized or rendered unavailable by the lime. Increase in pH value does not seem to be a sufficient explanation for the effect produced. On sandy soils such as are used for tobacco culture there is a much lower buffering effect than on heavier soils, hence the pH value may be raised considerably by only relatively light applications of ground limestone. This has occurred in the instance reported in this paper. Determination of the pH value following liming is the simplest method of determining whether a condition of low boron availability may have been reached.

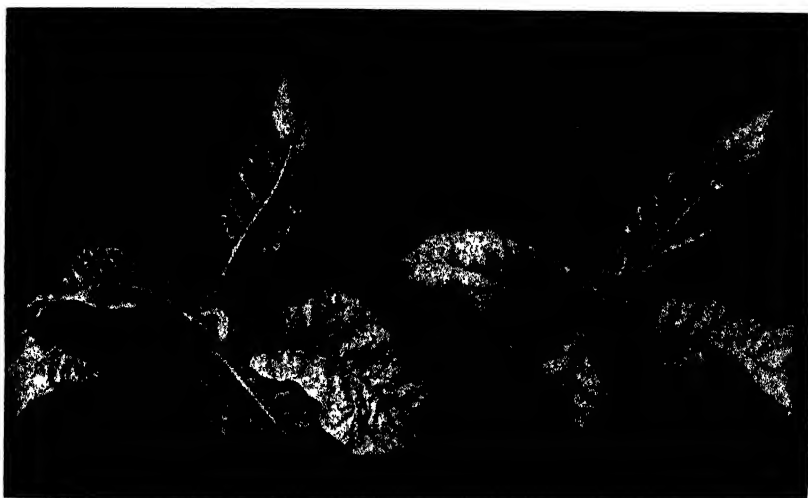


FIG. 5.—Heads of affected plants: note death or twisting of growing points. Left: 2 tons limestone per acre. Right: 1 ton limestone per acre.

At the Tobacco Research Station it was very interesting to note that the detrimental effect on growth was not observed until the season following the application of the ground limestone. By that time, too, with the cultivation that had been done between the two seasons, the limestone would have been very thoroughly mixed with the soil, so that reaction with the exchangeable base complex would have been extensive.

The symptoms shown by the plants on the limed areas correspond very closely with those recorded by van Schreven(3) as occurring on boron-deficient plants grown under sand culture. As shown in Figs. 3 and 4, curling of the leaves and death of the growing point with the development of secondary growth were found. Chlorosis and crinkling of the leaves is distinctly visible in Fig. 5. Bending over of the growing point is also marked. This is reminiscent of "Topsiekte," an ailment of tobacco in Sumatra, which has been attributed to boron deficiency. The combination of symptoms, together with the low boron content of the tobacco leaves, indicate that the ailment at Umukuri was due to boron deficiency. This deficiency was



induced by the applications of ground limestone being somewhat excessive considering the texture of the soil. A warning may therefore be issued that if it is desired to use ground limestone on the sandy soils used for tobacco culture in the Nelson district, the applications should be relatively light, not exceeding  $\frac{1}{2}$  ton per acre in any one season. Frequent use of lime on such soils may also be attended by risk of inducing a low availability of the boron in the soil in later seasons.

#### ACKNOWLEDGMENTS

The author wishes to thank Miss J. Watson for determining the exchangeable bases and the pH values of the soil samples. Thanks are gratefully offered to Mr. L. G. Copp for drawing attention to the affected plants and to Mr. R. Thomson, Director of the Tobacco Research Station, for details of cultural operations.

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## SOIL DISINFECTION

### V. COMPARATIVE EFFICACY OF SOIL FUMIGANTS FOR CONTROL OF GRASS-GRUB (*ODONTIA ZEALANDICA* WHITE)

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[Received for publication, 7th August, 1946]

#### Summary

Trials of the soil fumigants, chloropicrin, Shell D-D, and Iscobrome I and II in comparison with carbon disulphide showed that they are all of value in the control of grass-grub.

Chloropicrin and Iscobrome II appear to be effective at lower dosages than do the others. Carbon disulphide, to give equivalent control, seems to require a higher rate of application than that of any of the other fumigants.

#### INTRODUCTION

EXPERIMENTS reported by Cottier (1932, 1934) showed carbon disulphide to be the most effective fumigant then available for eradicating grass-grub from infested soil. With the introduction of chloropicrin and Shell D-D as soil fumigants it appeared desirable to compare the efficiency of these with that of carbon disulphide. Results of exploratory trials are recorded in this paper.

#### EXPERIMENTAL METHODS AND RESULTS

Treatments were applied either to pots filled with soil containing grass-grub larvæ or else to infested lawns. In the former case known numbers of larvæ were placed in the pots before treatment. In the latter, samples of soil were taken from each plot before treatment, and the larvæ in each sample counted. The figures thus obtained were used as the basis for calculating percentage survivals after treatment.

Fumigants employed were—

- (a) Carbon disulphide (chemically pure) :
- (b) Shell D-D, a mixture of 50 : 50 dichloropropane and dichloropropene :
- (c) Chloropicrin (chemically pure) :
- (d) Iscobrome I—15 per cent. methyl bromide and 85 per cent. xylol :
- (e) Iscobrome II—25 per cent. chloropicrin, 15 per cent. methyl bromide, and 60 per cent. xylol.

These materials were introduced into holes dibbled in the soil to a depth of 6 in., the requisite quantity of liquid being measured from a burette. Alternatively in infested lawns, an instrument\* specially designed to inject the required quantity directly into the soil was used. Points of injection in lawns were spaced 1 ft. apart. Carbon disulphide, Shell D-D, and chloropicrin were also applied as emulsions in water, prepared by the use of Wetsit, a proprietary emulsifying agent. Emulsions were prepared by using a stock solution in proportions of 1 ml. Wetsit to 10 ml. of chloropicrin and 1 ml. Wetsit to 5 ml. of Shell D-D and carbon disulphide. These solutions were diluted with water to give dosages chosen for treatments. In each instance 1 gallon of diluted emulsion was used per square yard and applied with a watering-can. A further gallon of water was applied after the emulsions had soaked into the soil. Results are expressed as percentage survivals, and, because of the small numbers of larvæ used, analyses of variance have been made on equivalent angles.

### Experiment I

In June, 1945, forty-four 10 in. flower-pots were filled with soil, and into each were placed twenty larvæ. Chemicals were introduced the following day, the holes then being covered immediately, and half a pint of water applied to each pot. After forty-eight hours the larvæ were sifted out and placed on the surface soil of each pot. Those larvæ which were unable to burrow into the soil were counted as dead. Treatments were replicated four times, there being one pot per replicate. Results are given in Table I.

TABLE I.—CONTROL OF GRASS-GRUB BY FUMIGANTS IN BARE SOIL

Treatments.	Millilitres per Cubic Foot.	Mean Percentage Survival out of Twenty Grubs.	Mean Equivalent Angles.
Check .. ..	..	100.00	..
Chloropicrin .. ..	2.0	45.00	42.00
" .. ..	4.0	0.00	..
Iscobrome I .. ..	2.0	15.00	21.95
" .. ..	4.0	0.00	..
Iscobrome II .. ..	2.0	2.50	8.05
" .. ..	4.0	0.00	..
Shell D-D .. ..	2.0	50.00	44.95
" .. ..	4.0	0.00	..
Carbon disulphide .. ..	4.0	12.50	20.37
" .. ..	8.0	2.50	8.05
Standard error .. ..	..	..	3.93
Difference for significance at 5 per cent. level .. ..	..	..	11.67

\* The instrument referred to was designed by Mr. L. Wright, of the Plant Diseases Division, and is still under test.

All treatments as compared with the check gave a significant control. Chloropicrin and Shell D-D at the lower dosage rates were the least effective treatments, while Iscobrome II gave the best results. The grubs were found close to the bottom of pots. The efficacy of higher dosages may possibly be explained by their greater rate of diffusion.

### Experiment II

Twenty-eight 8 in. flower-pots were sown with rye-grass seed, and twenty larvæ were placed in each pot. After thirty days, by which time the grass had grown, the chemicals were applied as emulsions at the rate of 200 ml. per pot (equivalent to approximately 1 gallon per square yard). Larvæ were examined ten days later. Results, based on four replications, with one pot per replicate, are given in Table II.

TABLE II.—CONTROL OF GRASS-GRUB BY EMULSIFIED FUMIGANTS IN SOIL SOWN WITH RYE-GRASS

Treatments.	Millilitres per Cubic Foot.	Mean Percentage Survival out of Twenty Grubs.	Mean Equivalent Angles.
Check .. ..	..	82.50	66.20
Chloropicrin .. ..	0.37	3.75	10.57
" .. ..	0.75	0.00	..
Shell D-D .. ..	0.37	20.00	26.35
" .. ..	0.75	2.50	7.30
Carbon disulphide .. ..	1.50	37.50	37.45
" .. ..	3.00	20.00	26.32
Standard error .. ..	..	..	4.07
Difference for significance at 5 per cent. level	..	..	12.09

The numbers of surviving larvæ were significantly reduced by all treatments. Chloropicrin was the most effective, giving significantly lower survivals than carbon disulphide at both dosage rates. Shell D-D at the higher dosage also showed significant improvement compared with carbon disulphide. Chloropicrin caused damage to rye-grass leaves, but did not affect later growth. The grubs were found close to the surface amongst the turf.

### Experiment III

A lawn infested with grass-grub was treated on 13th June, 1945, with chloropicrin, Shell D-D, and carbon disulphide. Prior to treatment populations were estimated by lifting four samples of soil in 8 in. cubes from each plot. Chemicals were applied to plots 25 sq. ft. in area, either in dibbled holes from a burette or as emulsions watered on to the surface. Before applying emulsions the turf was loosened lightly with a garden fork. Treatments were randomized in each of two blocks, emulsions in one and injections in the other. The figures obtained before application of treatments were used as a basis for the calculation of percentage survivals, which were taken several weeks later by the same sampling methods. Results are given in Table III.

TABLE III.—CONTROL OF GRASS-GRUB IN A LAWN, SEASON 1945

Treatments.	Millilitres per Square Foot.	Mean Number of Grubs.	Mean Percentage Survival.	Mean Equivalent Angles.
Injections applied in dibbled holes— Check .. .. Chloropicrin .. .. Shell D-D .. .. Carbon disulphide .. ..	  2.0 2.0 5.0	 5.75 5.50 4.50 5.25	 87.50 21.12 16.66 29.40	 71.32 26.90 22.07 32.55
Standard error .. ..	..	..	..	7.32
Difference for significance at 5 per cent. level	..	..	..	23.39
Emulsions— Check .. .. Chloropicrin .. .. Shell D-D .. .. Carbon disulphide .. ..	  2.0 2.0 5.0	 6.50 4.00 3.00 4.25	 94.06 33.33 72.91 70.83	 76.42 35.07 60.52 57.87
Standard error .. ..	..	..	..	3.30
Difference for significance at 5 per cent. level	..	..	..	10.53

Infestation was significantly reduced by all injection treatments. Because of small numbers of larvæ in each sample the difference required for significance was too high to show comparative efficacy of treatments. Emulsions also reduced infestation, but, compared with injections, the degree of control was poor. Chloropicrin was significantly more effective than Shell D-D and carbon disulphide. Treatments did not cause any apparent damage to the grass. New growth of grass developed more rapidly on the treated plots than on checks, increased vigour being most pronounced where chemicals were injected.

#### Experiment IV

On 31st May, 1946, a lawn infested with grass-grub was treated by injecting chloropicrin, Shell D-D, Iscobrome I and II, and carbon disulphide with the soil injector. Populations of larvæ were estimated as described in experiment III immediately before treatment and again on 21st June. Each plot was 63 sq. ft. in area, and treatments were randomized in each of four blocks. Results are given in Table IV.

TABLE IV.—CONTROL OF GRASS-GRUB IN A LAWN, SEASON 1946

Treatments.	Millilitres per Square Foot.	Mean Number of Grubs.	Mean Percentage Survival.	Mean Equivalent Angles.
Check .. .. Chloropicrin .. .. Carbon disulphide .. .. Iscobrome I .. .. Iscobrome II .. .. Shell D-D .. ..	 2.0 5.0 2.0 2.0 2.0	 16.50 13.00 15.75 15.00 12.75 9.75	 78.60 11.14 21.36 21.25 16.03 29.74	 63.77 18.65 27.17 26.32 21.72 32.40
Standard error .. ..	..	..	..	5.72
Difference for significance at 5 per cent. level	..	..	..	17.16

Although all treatments gave significant reduction in infestation compared with the checks, they did not differ significantly amongst themselves. One plot in each of the chloropicrin and Iscobrome II treatments showed percentage survivals that were abnormally high. It seems probable that improved methods of sampling would show these two treatments in a more favourable light. Chloropicrin and Iscobrome II caused wilting of the grass adjacent to points of injection. Within a few weeks all treatments showed marked improvement in growth of grass compared with checks.

#### CONCLUSIONS

All fumigants tested gave results in control of grass-grub which justify further experiments. Because of variation in results it seems desirable that the method of application and sampling will also have to be investigated further. The present trials are comparative only and were not designed to ascertain optimum dosages for control. Results suggest that dosage rates for carbon disulphide would require to be higher than for any of the other test fumigants in order to obtain equivalent control. Chloropicrin and Iscobrome II appear likely to give control at lower dosages than Shell D-D and Iscobrome I.

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## THE EFFECT OF VARIOUS COBALT COMPOUNDS ON THE COBALT CONTENT OF A NELSON PASTURE

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#### Summary

Using 1 cwt. per acre of ground limestone as a spreading agent, the sulphate, hydroxide, carbonate, and phosphate of cobalt were applied to a cobalt-deficient pasture at Sherry River. All four compounds effectively increased the cobalt content of the pasture over a period of sixteen months.

#### INTRODUCTION

THE use of cobalt salts for the prevention and cure of bush sickness and similar diseases has become established in New Zealand and other countries. In general, the chloride or sulphate has been the salt chosen for use in fertilizers, licks, and drenches. It is of interest, however, to determine, if possible, the effect of other compounds of cobalt, particularly those which are soluble in water to only a slight degree, on the intake of cobalt by pasture plants. Such a test has been made and is reported in this paper.

## EXPERIMENTAL

The pasture, consisting mainly of perennial rye-grass and white clover, was on a sandy loam derived from granite at Sherry River, Nelson. It was laid down only a few years before the experiment began and had received ground limestone and superphosphate treatment. In the 1938-39 season it was cut for hay and was afterwards grazed. Experimental work began on the pasture in the 1939-40 season when replicated plots received superphosphate at the rate of 2 cwt. per acre. Another application of superphosphate at this rate was made on 21st August, 1941. Cobalt compounds, at rates equivalent to 5 oz. and 10 oz. of hydrated cobalt sulphate per acre, together with 1 cwt. ground limestone per acre as a carrier for the cobalt compound, were applied on 11th December, 1941, on plots 5 yards square, replicated three times. The cobalt compounds used were the sulphate, hydroxide, and carbonate. Cobalt phosphate (octohydrate), owing to an error, was applied at rates equivalent to 8.25 oz. and 16.5 oz. hydrated sulphur per acre. Rain fell on the day following application of the mixtures. In the following season, superphosphate at 2 cwt. per acre was applied on 15th October, 1942. Sampling was done periodically by careful hand-picking, special care being taken to avoid contamination of the material by soil. In spite of this, some of the analytical data suggest that contamination occurred. Definite occurrences are shown by blank spaces in Table I, but other cases probably occurred as indicated by certain aberrant data in this table. On each occasion after sampling the experimental area was grazed by sheep for a few days and then closed up without any stock on it until the next time of sampling.

The first samples were obtained on 8th January, 1942, four weeks after the cobalt was applied. Thereafter sampling was done at approximately monthly intervals, except over the winter of 1942.

TABLE I.—EFFECT OF COBALT COMPOUNDS ON COBALT CONTENT OF A SHERRY RIVER PASTURE

(Cobalt content expressed as parts per million of cobalt on the dry basis)

Date of Sampling.	Control (No Cobalt).	Limestone only	Cobalt Sulphate.		Cobalt Hydroxide.		* Cobalt Carbonate		Cobalt Phosphate	
			1.*	2.*	1.*	2.*	1.*	2.*	1.*	2.*
8/1/42	0.061	..	0.27	0.37	0.21	0.37	0.73	0.93	0.69	1.34
4/2/42	0.039	0.037	0.14	0.16	0.13	0.14	0.23	0.20	0.17	0.41
27/2/42	0.031	0.028	0.080	0.067	0.041	0.086	0.083	0.059	0.083	0.084
13/4/42	0.040	0.031	0.064	0.12	0.038	0.091	0.052	0.089	0.067	0.16
14/5/42	0.057	0.042	0.108	0.14	0.085	0.135	..	0.109	0.134	0.134
16/10/42	0.078	0.074	0.118	0.132	0.181	0.128	0.096	0.15	0.105	0.152
17/11/42	0.052	0.045	0.078	0.081	0.092	0.105	0.075	0.104	0.108	0.16
16/12/42	0.056	0.038	0.081	0.106	0.057	0.079	0.069	0.079	0.053	0.14
19/1/43	0.048	0.075	0.096	0.14	0.072	0.11	0.12	0.11	0.078	0.14
2/3/43	0.047	0.090	0.12	0.107	0.079	0.107	0.084	0.09	0.10	0.13
6/4/43	0.036	0.055	0.050	0.087	0.052	0.068	0.056	0.061	0.062	0.11

\* 1 = lower rate of application ; 2 = higher rate of application

Results of determinations of cobalt content on the dry-matter basis are given in Table I. The data suggest that over the whole period of the experiment cobalt carbonate, cobalt hydroxide, and cobalt sulphate were of approximately equal value in increasing the cobalt content of this Sherry

River pasture. In the early stages the carbonate was slightly more effective. The heavier rate of application of the phosphate is reflected in the higher cobalt contents shown for this compound. While, in general, the double rates of application of each compound show the higher figures for cobalt content, this is not without exception; contamination with soil may explain these abnormal cases. It is to be noted, also, that the May and October samples show high values, even for those plots not receiving cobalt. While the possibility of contamination cannot be excluded, the increases are so consistent as to suggest that the effect is a real one, due to seasonal conditions. Even after sixteen months from the date of application all the compounds were showing appreciable effects, this being more marked with the double rates of application. Ground limestone has exerted no great effect on the intake of cobalt and thus would not seem to have interfered with the action of the cobalt compounds.

### DISCUSSION

In an investigation of the use of various carriers for cobalt sulphate, Maunsell and Simpson(1) used several types of fertilizer materials and limestone on pastures at Mamaku and Ruakura, in the North Island. Cobalt sulphate at the rates of 5 oz. and 10 oz. per acre were used, with the carrier usually at the rate of 1 cwt. per acre. They concluded that all the carriers were satisfactory and that there appeared to be no worth-while difference in the effects of the two rates of application after two to four months according to the location of the experimental area. Their investigation covered the period July, 1941, to February, 1942. In the experiment at Sherry River, described in the present paper, the period of examination was longer and differed in the use of so-called insoluble cobalt compounds as well as the soluble sulphate; only the one carrier, ground limestone, was used. This limestone carried approximately 0.2 p.p.m. of cobalt. Satisfactory increases in cobalt content of the pasture followed from the applications of both the soluble sulphate and the insoluble hydroxide, carbonate, and phosphate. It is apparent, therefore, that the solubility of these three compounds is sufficiently great to allow of an appreciable uptake of cobalt by the pasture plants. Mellor(2) quotes data giving the solubility of cobaltous hydroxide as 3.18 mg. per litre and of cobaltous carbonate as 27.3 mg. per litre. The sample of cobalt phosphate octohydrate used at Sherry River was found(3) to have a solubility of 5.1 mg. per litre as anhydrous phosphate or of 2.45 p.p.m. of cobalt (Co). These values appear to be high enough to explain the markedly beneficial effect of these compounds in raising the cobalt content of the pasture. This experiment therefore indicates that the hydroxide, carbonate, or phosphate of cobalt may be used with satisfaction for pasture top-dressing on cobalt-deficient areas in place of the soluble chloride or sulphate.

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## MINERAL DEFICIENCY OF APPLE-LEAVES: DISTRIBUTION OF MAGNESIA, POTASH AND LIME IN THE LEAVES OF YOUNG SHOOTS

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### Summary

An examination of the mineral composition of the current season's growth on Cox's Orange apple-trees from three different localities in the Nelson district showed that the leaves varied in mineral content with position on the leader, and that where magnesium or potassium deficiencies occurred the element in low supply tended to be highest in the young leaves near the growing point when calculated as a percentage of the dry matter. In all cases the young growth had a lower calcium content than older leaves on the leader shoot.

Changes in mineral content of the leader leaves are shown to have taken place during the season. Calcium tended to increase with age of the leaf. Trees deficient in either magnesium or potassium showed a decrease in the total content per leaf of the deficient element in the lower leaves of the leader as the season progressed, indicating a withdrawal, probably to supply the growing point of the shoot. The development of the fruit may also have made demands on these lower leader leaves.

The appearance of magnesium-deficiency symptoms in Cox's Orange and Jonathan leaves was associated with a low content of this element (less than 0.14 per cent. MgO), but could not be correlated with any particular percentage of magnesium in the whole leaf.

### INTRODUCTION

MAGNESIUM deficiency of apple-trees in the Nelson district has been identified and reported in previous papers(1, 2). Further investigations into the distribution of magnesium, calcium, and potassium in the apple leader, undertaken mainly in order to develop a suitable leaf-sampling technique, are described in this paper. The data include analyses of leaves and wood from different parts of the leader shoot and also figures showing the changes that took place in the magnesium, potassium, and calcium contents of the apple-leaves as the season progressed.

### METHODS OF ANALYSIS

Magnesium was determined by the gravimetric phosphate method for large samples and by the 8-hydroxy-quinoline method(3) for small samples. Calcium was precipitated as oxalate. The potash was estimated by the perchloric acid method.

### THE DISTRIBUTION OF MAGNESIUM, CALCIUM, AND POTASSIUM IN DIFFERENT PARTS OF THE LEADER SHOOT†

On 3rd December, 1940, fifty Cox's Orange leaders were collected from an orchard on the Moutere Hills soil type at Tasman, where acute magnesium deficiency symptoms were known to occur, and the same number from an

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† "Leader" is used to denote the current season's terminal or lateral growth.



orchard on Stoke loam which was well supplied with magnesium but which had a relatively low potash status. In both cases five leaders from each of ten trees were sampled. The leaders were taken early in the season before defoliation had begun. They were divided approximately into quarters according to the number of leaves, and corresponding leaf and wood samples were bulked. Each quarter consisted of 3 to 5 leaves. The terminal bud and the leaf petioles were included with the wood. In the Tasman samples slight scorch, typical of magnesium deficiency, was appearing in some of the leaves from the lower half of the leaders. Spray residues were removed, where necessary, by wiping with a dry cloth. Analyses for the composite samples are given in Table I.

TABLE I.—ANALYSIS OF LEAVES AND WOOD FROM DIFFERENT PARTS OF THE LEADER, COX'S ORANGE VARIETY (SAMPLED 3RD DECEMBER, 1940)  
(Expressed as percentage of the dry matter)

Position of Sample.			Soluble Ash.	CaO.	MgO.	K <sub>2</sub> O.
(a) <i>Tasman</i>						
Leaves—						
Lowest quarter	..	..	6.85	1.68	0.21	2.18
Second quarter	..	..	6.61	1.16	0.19	2.63
Third quarter	..	..	7.07	1.11	0.32	2.95
Top quarter	..	..	6.22	0.94	0.40	2.38
Wood—						
Lowest quarter	..	..	4.99	1.37	0.15	1.51
Second quarter	..	..	5.19	1.36	0.17	1.58
Third quarter	..	..	6.73	1.56	0.27	2.31
Top quarter	..	..	8.81	1.73	0.62	3.18
(b) <i>Stoke</i>						
Leaves—						
Lowest quarter	..	..	6.29	2.20	0.69	0.80
Second quarter	..	..	4.73	1.38	0.55	0.87
Third quarter	..	..	4.56	1.18	0.60	0.88
Top quarter	..	..	4.60	0.92	0.58	1.26
Wood—						
Lowest quarter	..	..	4.50	1.61	0.32	0.73
Second quarter	..	..	4.12	1.36	0.25	0.79
Third quarter	..	..	5.16	1.59	0.39	1.12
Top quarter	..	..	6.89	1.77	0.83	1.92

From Table I it will be seen that there was considerable variation in mineral constituents according to position on the leader.

### Leaves

In each case the mineral in low supply was found to be most highly concentrated at the growing point of the shoot. Thus the magnesium in the leaves of the magnesium-deficient Tasman leaders increased from 0.21 and 0.19 per cent. MgO in the lower half to 0.40 per cent. in the top quarter, while potash showed no such accumulation in the youngest leaves. In the Stoke sample, on the other hand, the potash was low for all parts, but was highest in the top leaves, which contained 1.26 per cent. K<sub>2</sub>O, compared

with 0.80 to 0.88 per cent. in the lower sections. The magnesium in this Stoke sample was relatively high and approximately constant irrespective of position on the leader. In both localities the calcium increased with age of the leaf.

### Wood

In the wood all three elements were highest in the top quarter, this being most noticeable in the magnesium figures for the magnesium-deficient Tasman sample, where there was an increase from 0.15 and 0.17 per cent. MgO in the lower half to 0.62 per cent. in the top wood. There was also a very marked increase in potash content in the upper portion of the Stoke sample.

### CHANGES IN THE MINERAL CONTENT OF LEAVES THROUGHOUT THE SEASON

In order to determine to what extent magnesium-deficient apple-trees show a low magnesium content in their leaves before visible symptoms appear and also to determine the change taking place in this and other minerals with ageing of the leaf, a series of samples was taken throughout the season from October to early in March when defoliation had become widespread on the Tasman orchard. As before, five leaders from each of the same ten trees were taken. The five lowest leaves were taken at the beginning of the experiment, and, as the shoot grew, the five lowest and the four top leaves, and finally, from November onwards, five leaves next to the lowest ones were also sampled. By 3rd December magnesium-deficiency symptoms were appearing at Tasman and samples of the ten lowest leaves contained a number of scorched leaves on this and later dates of sampling. The results are given in Table II.

TABLE II.—CHANGES IN THE MINERAL CONTENT OF COX'S ORANGE LEADER LEAVES THROUGHOUT THE SEASON (1940-41)

(Expressed as percentage of dry matter)

Position on Leader.			Date of Sampling.	Soluble Ash.	CaO.	MgO.	K <sub>2</sub> O.
(a) <i>Tasman</i>							
Five lowest leaves	..	..	10/10/40	6.54	1.38	0.33	2.29
Five lowest leaves	..	..	23/10/40	6.51	1.51	0.28	2.24
Four top leaves	..	..	..	5.62	0.84	0.26	2.36
Five lowest leaves	..	..	6/11/40	6.82	1.66	0.28	2.23
Four top leaves	..	..	..	5.99	0.85	0.29	2.46
Five lowest leaves	..	..	20/11/40	7.25	1.85	0.25	2.24
Next five leaves	..	..	..	6.74	1.11	0.22	2.72
Four top leaves	..	..	..	6.92	1.36	0.43	2.33
Five lowest leaves	..	..	3/12/40	6.77	1.63	0.20	2.23
Next five leaves	..	..	..	6.39	1.06	0.20	2.64
Four top leaves	..	..	..	6.11	0.97	0.41	2.46

TABLE II.—CHANGES IN THE MINERAL CONTENT OF COX'S ORANGE LEADER LEAVES  
THROUGHOUT THE SEASON (1940-41)—*continued*  
(Expressed as percentage of dry matter)

Position on Leader.			Date of Sampling.	Soluble Ash.	CaO.	MgO.	K <sub>2</sub> O.
(a) <i>Tasman</i> —continued							
Four lowest leaves*	..	..	18/12/40	7.50	1.65	0.18	2.59
Next four leaves ..	..	..	"	7.23	1.30	0.22	2.80
Four top leaves ..	..	..	"	6.98	0.87	0.39	2.80
Five lowest leaves	..	..	4/1/41	7.92	2.06	0.17	2.43
Next five leaves ..	..	..	"	7.29	1.52	0.15	2.60
Four top leaves ..	..	..	"	5.61	0.67	0.41	2.30
Five lowest leaves	..	..	23/1/41	7.45	1.92	0.17	2.34
Next five leaves ..	..	..	"	6.57	1.37	0.16	2.37
Four top leaves ..	..	..	"	4.98	0.74	0.41	2.03
Five lowest leaves	..	..	4/3/41	8.94	2.55	0.16	2.50
Next five leaves ..	..	..	"	8.03	2.09	0.15	2.40
Four top leaves ..	..	..	"	5.92	1.47	0.38	1.86
(b) <i>Stoke</i>							
Five lowest leaves	..	..	10/10/40	5.78	1.66	0.53	1.21
Five lowest leaves	..	..	23/10/40	6.20	2.05	0.63	0.98
Four top leaves ..	..	..	"	4.48	0.86	0.36	1.45
Five lowest leaves	..	..	6/11/40	6.74	†	0.70	0.93
Four top leaves ..	..	..	"	5.15	..	0.46	1.56
Five lowest leaves	..	..	20/11/40	5.88	..	0.63	0.82
Next five leaves ..	..	..	"	4.36	..	0.49	0.99
Four top leaves ..	..	..	"	4.96	..	0.57	1.40
Five lowest leaves	..	..	4/12/40	6.37	..	0.70	0.80
Next five leaves ..	..	..	"	4.64	..	0.55	0.89
Four top leaves ..	..	..	"	4.87	1.06	0.61	1.29
Four lowest leaves*	..	..	18/12/40	6.03	2.08	0.66	0.75
Next four leaves ..	..	..	"	5.04	1.46	0.60	0.86
Four top leaves ..	..	..	"	4.67	1.16	0.67	1.00
Five lowest leaves	..	..	4/1/41	6.54	2.45	0.69	0.61
Next five leaves ..	..	..	"	4.99	1.60	0.58	0.67
Four top leaves ..	..	..	"	4.28	1.08	0.79	0.78
Five lowest leaves	..	..	23/1/41	6.53	2.56	0.75	0.53
Next five leaves ..	..	..	"	5.14	1.76	0.61	0.60
Four top leaves ..	..	..	"	4.43	1.23	0.80	0.77
Five lowest leaves	..	..	4/3/41	7.13	2.86	0.80	0.52
Next five leaves ..	..	..	"	5.91	2.21	0.71	0.59
Four top leaves ..	..	..	"	5.52	1.77	1.02	0.70

\* On 18th December four leaves were taken instead of five for the two lower samples. † Owing to contamination of some of the leaves with spray, the calcium figures were omitted.

### *Tasman*

**Magnesium.**—It will be seen that there was a gradual decrease in the magnesium content of the five lowest leaves from 0.33 per cent. MgO when the leader was very young to 0.16 per cent. in March when scorch and defoliation were prevalent. By early December the figure had dropped to

0.20 per cent., and from then on only a slow decrease in magnesium took place. The next five leaves had an initial figure of 0.22 per cent. MgO on 20th November, though the leaves corresponded approximately to the sample taken as the four top leaves on 23rd October, which had a figure of 0.26 per cent. By 3rd December there was a decrease to 0.20 per cent., with a further fall to 0.15 per cent. in early January. The sample gave the same figure on 4th March and was slightly below the five lowest leaves in magnesium content for the last three samplings, a fact of interest when it is realized that the deficiency symptoms in Cox's Orange often appear at about the fifth or sixth leaf, while the lower ones remain free from leaf-burn.

As shown by the data in Table I, the young leaves had a much higher magnesium content than the ones on the lower part of the leader. There was a rise from 0.26 per cent. MgO in October to 0.43 per cent. towards the end of November, later samples giving approximately this last figure.

*Potassium.*—The potassium content did not vary much with position or age of the leaf, except that towards the end of the season there was a decrease in the amount of potassium in the top leaves. The trees had had fairly heavy potash manuring and there was no sign of a deficiency of this element.

*Calcium.*—Calcium showed a tendency to increase with the age of the leaf.

#### Stoke

*Magnesium.*—The magnesium content of the lower leaves from this locality was several times that of corresponding Tasman samples during most of the period of the experiment. The figure varied between 0.53 and 0.80 per cent. MgO. Leaves at the top did show a slightly higher content than the ten bottom leaves, but not until January, and then not to a very marked degree. It is very probable that the figures for magnesium were abnormally high owing to the low potash supply in this orchard.

*Potassium.*—The low potassium content of the Stoke leaders compared with those from Tasman was very noticeable. For the five bottom leaves the initial figure of 1.21 per cent.  $K_2O$  dropped as low as 0.52 per cent. in January, when the corresponding Tasman leaves contained 2.34 per cent. The four top leaves gave 1.45 and 1.56 per cent.  $K_2O$  for the first two samplings, after which there was a steady decrease to 0.70 per cent.  $K_2O$  when the last sample was taken in March. The figures for the Tasman samples were 2.36, 2.46, and 1.86 per cent.  $K_2O$  at these three dates of sampling. As previously noted (Table I), the potash, which was in short supply in the Stoke soil, was higher in the top leaves than in those on the lower part of the leader. This was most noticeable in the early summer when rapid growth of the leader was taking place.

*Calcium.*—As in the Tasman samples, there was a tendency for increase in calcium content with age of the leaf, especially towards the end of the season. The calcium figures were, on the whole, higher than those from Tasman.

Leaders from the magnesium-deficient Tasman orchard and also from an orchard in Waimea West on the same soil type but at the time showing no symptoms of either potassium or magnesium deficiency were sampled in the following season, beginning in December, 1941.

At Tasman, magnesium-deficiency symptoms were appearing at the time of the January sampling and some scorched leaves were included in the lower samples in both January and February.

The results are given in Table III.

TABLE III.—CHANGES IN THE MINERAL CONTENT OF COX'S ORANGE LEADER LEAVES THROUGHOUT THE SEASON (1941-42)

(Results expressed as percentage of the dry matter)

Position on Leader.			Date of Sampling.	Soluble Ash.	CaO.	MgO.	K <sub>2</sub> O.
<i>(a) Tasman</i>							
Five lowest leaves	..	..	9/12/41	5.89	1.33	0.19	1.89
Next five leaves	..	..	"	5.67	0.92	0.22	2.88
Four top leaves	..	..	"	5.17	0.73	0.42	1.97
Five lowest leaves	..	..	12/1/42	7.38	1.68	0.14	2.44
Next five leaves	..	..	"	6.98	1.40	0.17	2.50
Four top leaves	..	..	"	5.98	0.69	0.33	2.45
Five lowest leaves	..	..	16/2/42	8.03	1.99	0.14	2.40
Next five leaves	..	..	"	..	1.54	0.14	2.09
Four top leaves	..	..	"	5.38	0.78	0.40	2.06
Four top leaves	..	..	16/3/42	5.47	0.79	0.41	2.18
Four top leaves	..	..	21/4/42	4.40	1.28	0.50	0.82
<i>(b) Waimea West</i>							
Five lowest leaves	..	..	24/12/41	4.59	0.97	0.32	1.48
Next five leaves	..	..	"	4.51	0.68	0.30	1.77
Four top leaves	..	..	"	4.88	0.73	0.41	1.89
Five lowest leaves	..	..	12/1/42	5.14	1.03	0.29	1.72
Next five leaves	..	..	"	4.95	0.77	0.28	1.88
Four top leaves	..	..	"	4.90	0.58	0.41	1.99
Five lowest leaves	..	..	16/2/42	5.23	1.15	0.28	1.57
Next five leaves	..	..	"	5.08	1.00	0.28	1.60
Four top leaves	..	..	"	4.34	0.74	0.54	1.37
Five lowest leaves	..	..	16/3/42	5.97	1.36	0.30	1.82
Next five leaves	..	..	"	5.35	1.08	0.29	1.86
Four top leaves	..	..	"	4.37	0.77	0.44	1.38
Four top leaves	..	..	21/4/42	4.67	1.07	0.42	1.27

In the Tasman samples the same trends were apparent for magnesium as in the previous season, magnesium being low in the two sets of lower leaves and remaining comparatively high in the top leaves throughout the sampling period. By 16th March defoliation was too far advanced for more than the top sample to be taken. It was interesting to note that by 21st April, browning was present even in the top leaf sample, but it is possible that this browning was due to natural ageing rather than to magnesium deficiency. On this date the magnesium figure (0.50 per cent. MgO) was well above that normally associated with deficiency symptoms. The potash remained high, except in the last sample in April. As before, lime was lowest in the youngest leaves.

The magnesium content of the Waimea West samples showed little change throughout the period of the experiment, being about 0.30 per cent. MgO for the lower leaves and 0.41 to 0.54 per cent. for the top leaves. The potash was not so high as in the Tasman samples, but the figures were considerably higher than those reported for the Stoke area in Table II.

## WEIGHT OF CALCIUM, MAGNESIUM, AND POTASSIUM PER LEAF

The data in Tables II and III are expressed in Tables IV and V respectively as average weights of CaO, MgO, and K<sub>2</sub>O per leaf. The average weight of dry matter per leaf at each time of sampling is also given.

TABLE IV.—CHANGES IN THE MINERAL CONTENT OF COX'S ORANGE LEADER LEAVES THROUGHOUT THE SEASON (1940-41)

(Average weights of dry matter, CaO, MgO, and K<sub>2</sub>O, per leaf)

Position on Leader.			Date sampled.	Dry Matter (g.).	CaO (mg.).	MgO (mg.).	K <sub>2</sub> O (mg.).
<i>(a) Tasman</i>							
Five lowest leaves	..	..	10/10/40	0.128	1.77	0.42	2.93
Five lowest leaves	..	..	23/10/40	0.182	2.75	0.51	4.09
Four top leaves	..	..	"	0.149	1.25	0.39	3.50
Five lowest leaves	..	..	6/11/40	0.234	3.88	0.65	5.21
Four top leaves	..	..	"	0.110	0.94	0.32	2.71
Five lowest leaves	..	..	20/11/40	0.269	4.98	0.67	6.03
Next five leaves	..	..	"	0.223	2.48	0.49	6.07
Four top leaves	..	..	"	0.098	1.33	0.42	2.28
Five lowest leaves	..	..	3/12/40	0.282	4.60	0.57	6.30
Next five leaves	..	..	"	0.218	2.31	0.44	5.74
Four top leaves	..	..	"	0.107	1.04	0.44	2.63
Four lowest leaves	..	..	18/12/40	0.256	4.22	0.46	6.62
Next four leaves	..	..	"	0.162	2.11	0.36	4.55
Four top leaves	..	..	"	0.117	1.02	0.46	3.27
Five lowest leaves	..	..	23/1/41	0.216	4.15	0.37	5.06
Next five leaves	..	..	"	0.169	2.32	0.27	4.01
Four top leaves	..	..	"	0.122	0.89	0.49	2.47
Five lowest leaves	..	..	4/3/41	0.284	7.25	0.45	7.11
Next five leaves	..	..	"	0.199	4.15	0.30	4.77
Four top leaves	..	..	"	0.144	2.12	0.55	2.68
<i>(b) Stoke</i>							
Five lowest leaves	..	..	10/10/40	0.130	2.16	0.69	1.57
Five lowest leaves	..	..	23/10/40	0.186	3.82	1.17	1.82
Four top leaves	..	..	"	0.112	0.96	0.40	1.63
Five lowest leaves	..	..	6/11/40	0.184	..	1.29	1.71
Four top leaves	..	..	"	0.090	..	0.41	1.40
Five lowest leaves	..	..	20/11/40	0.222	..	1.40	1.82
Next five leaves	..	..	"	0.171	..	0.84	1.69
Four top leaves	..	..	"	0.097	..	0.55	1.36
Five lowest leaves	..	..	4/12/40	0.225	..	1.57	1.80
Next five leaves	..	..	"	0.182	..	1.00	1.62
Four top leaves	..	..	"	0.125	1.32	0.76	1.61
Four lowest leaves	..	..	18/12/40	0.194	4.03	1.28	1.45
Next four leaves	..	..	"	0.168	2.45	1.01	1.44
Four top leaves	..	..	"	0.138	1.61	0.93	1.38
Five lowest leaves	..	..	23/1/41	0.211	5.39	1.58	1.09
Next five leaves	..	..	"	0.184	3.25	1.12	1.11
Four top leaves	..	..	"	0.113	1.39	0.91	0.87
Five lowest leaves	..	..	4/3/41	0.207	5.93	1.66	1.08
Next five leaves	..	..	"	0.168	3.71	1.19	0.99
Four top leaves	..	..	"	0.109	1.93	1.11	0.77

The weights of magnesium per leaf (Table IV) remained consistently low in the Tasman samples compared with those from Stoke, the most noticeable feature in the former being the slow rise in the four top leaves as the season progressed. The lower leaves dropped steadily in total magnesium content between 20th November and 23rd January, suggesting that magnesium was being drawn from these leaves to supply the top leaves of the growing shoot and possibly the fruit also. The difference in potassium content between the Tasman and Stoke samples was even more striking expressed as mg.  $K_2O$  per leaf than as percentage of dry matter, owing to the tendency for the Stoke leaves to be smaller. The Stoke weights of potash per leaf began to decrease in December, probably owing to a removal of potassium to supply the young growth and the fruit as in the case of magnesium in the Tasman leaders. The weight of calcium per leaf tended to increase with age of the leaf, particularly towards the end of the season, in both localities.

TABLE V.—CHANGES IN THE MINERAL CONTENT OF COX'S ORANGE LEADER LEAVES THROUGHOUT THE SEASON (1941-42)

(Average weights of dry matter, CaO, MgO, and  $K_2O$ , per leaf)

Position on Leader.	Date sampled.	Dry Matter (g.).	CaO (mg.).	MgO (mg.).	$K_2O$ (mg.).
<i>(a) Tasman</i>					
Five lowest leaves .. ..	9/12/41	0.249	3.31	0.47	4.71
Next five leaves .. ..	"	0.198	1.82	0.44	4.51
Four top leaves .. ..	"	0.055	0.40	0.23	1.08
Five lowest leaves .. ..	12/1/42	0.246	4.13	0.34	6.00
Next five leaves .. ..	"	0.182	2.55	0.31	4.55
Four top leaves .. ..	"	0.103	0.71	0.34	2.52
Five lowest leaves .. ..	16/2/42	0.213	4.24	0.30	5.11
Next five leaves .. ..	"	0.165	2.54	0.23	3.45
Four top leaves .. ..	"	0.097	0.76	0.39	2.00
Four top leaves .. ..	16/3/42	0.111	0.88	0.46	2.42
Four top leaves .. ..	21/4/42	0.122	1.56	0.61	1.00
<i>(b) Waimea West</i>					
Five lowest leaves .. ..	24/12/41	0.208	2.02	0.67	3.08
Next five leaves .. ..	"	0.206	1.40	0.62	3.65
Four top leaves .. ..	"	0.141	1.03	0.58	2.66
Five lowest leaves .. ..	12/1/42	0.231	2.38	0.67	3.97
Next five leaves .. ..	"	0.185	1.42	0.52	3.48
Four top leaves .. ..	"	0.130	0.75	0.53	2.59
Five lowest leaves .. ..	16/2/42	0.195	2.24	0.55	3.06
Next five leaves .. ..	"	0.189	1.89	0.53	3.02
Four top leaves .. ..	"	0.127	0.94	0.69	1.74
Five lowest leaves .. ..	16/3/42	0.197	2.68	0.59	3.59
Next five leaves .. ..	"	0.175	1.89	0.51	3.26
Four top leaves .. ..	"	0.125	0.96	0.55	1.73
Four top leaves .. ..	21/4/42	0.148	1.89	0.74	1.21

It will be seen from the above Table V that the weight of magnesium per leaf in the lower leaves of the Tasman leaders decreased during the period December to February, the figures being similar to those found in corresponding sampling periods during the 1940-41 season (Table IV).

As before, the top leaves rose in total magnesium content throughout the season. These trends were present in the Waimea West samples also, but only to a slight degree, and the magnesium content was higher for all positions than at Tasman. The potash results do not indicate a withdrawal of potassium from the leaves from December on, such as occurs at Stoke (Table IV). It seems probable, therefore, that the Waimea West trees are adequately supplied with potash, even though the amount taken up by the leaves is lower than in the Tasman orchard. The percentage of  $K_2O$  in the dry matter (Table III) is higher than the standard given by American workers as indicating an adequate potash uptake—viz., 1 per cent. K (equivalent to 1.2 per cent.  $K_2O$ ).

Calcium shows the same tendency to increase with age of the leaf, as is seen in previous tables.

#### MAGNESIUM CONTENT OF SCORCHED AND UNSCORCHED LEAVES ON THE SAME LEADER

In view of the variation in magnesium content that has been shown to occur in apple-leaves, an attempt was made to determine a critical figure for magnesium below which necrosis was likely to appear in the leaf. Both Jonathan and Cox's Orange leaders frequently show browning due to magnesium deficiency from the fourth, fifth, or sixth leaf upwards, with several healthy leaves at the bottom of the shoot. On 12th and 13th January, 1942, adjacent healthy and scorched leaves were obtained from the highest and lowest parts of the affected portion of a number of leaders of this type. Analyses of these samples are given in Table VI. A number of magnesium figures are also given for the highest scorched leaf and the healthy leaf above in a number of Jonathan leaders where magnesium deficiency had caused defoliation of the leaves on the lower part of the leader.

TABLE VI.—MAGNESIUM CONTENT OF ADJACENT SCORCHED AND HEALTHY LEAVES (Expressed as percentage of MgO on the dry-matter basis)

Variety, and Position on Leader.	Sample (1).	Sample (2).	Sample (3).	Sample (4).	Sample (5).	Sample (6).	Average.
<i>(a) Cox's Orange</i>							
Lowest scorched leaf ..	0.12	0.09	Trace	0.09	0.05	0.10	0.07
Healthy leaf below ..	0.13	0.15	0.08	0.13	0.09	0.16	0.12
Highest scorched leaf ..	0.09	0.003	0.05	0.005	0.05	0.13	0.05
Healthy leaf above ..	0.15	0.08	0.04	0.11	0.12	0.12	0.10
<i>(b) Jonathan</i>							
Lowest scorched leaf ..	0.05	..	..	..	..	..	..
Healthy leaf below ..	0.13	..	..	..	..	..	..
Highest scorched leaf ..	0.02	0.05	0.10	0.07	0.07	..	0.06
Healthy leaf above ..	0.20	0.04	0.10	0.10	0.13	..	0.11

It will be seen that all these leaves have magnesium figures below 0.17 per cent. MgO. In most cases the healthy leaves are higher in magnesium than the neighbouring scorched ones, the former averaging about twice the magnesium content of the latter.

However, it does not seem possible in either variety to fix a critical figure below which scorch appears. In the Cox's Orange variety, leaves which were still apparently healthy varied in magnesium content from 0.04 per cent. MgO to 0.16 per cent., while the scorched leaves ranged from a trace too small to be estimated by the 8-hydroxy-quinoline method to values



as high as 0.13 per cent. There was therefore considerable overlapping in the magnesium figures for apparently healthy and scorched leaves. Similar inconsistencies appeared for the Jonathan leaders. It may be pointed out that leaf-ash skeletons(4) have shown that the green tissue surrounding the necrotic area of an affected leaf can vary considerably from leaf to leaf in the amount of magnesium present. In some cases the amount is too small to give a colour with Titan yellow, and in others a distinct pink edging is seen. It is therefore likely that the appearance of browning cannot be associated with a particular magnesium content of the whole leaf. Using a sensitive Titan yellow method reported in a previous paper(5), it has now been found possible to estimate magnesium in smaller quantities of plant material than a single apple-leaf, and it is hoped to obtain more information by this method on the magnesium content of those parts of the leaf which usually develop necrosis. It is possible that some definite level of magnesium status may be associated with the appearance of scorch in these more localized areas.

#### DISCUSSION

Enough data have not yet been obtained to determine what is the normal content and distribution of calcium, magnesium, and potassium in an apple leader of the Cox's Orange variety.

For instance, neither the Stoke orchard nor the Waimea West orchard showed signs of magnesium deficiency, and might be considered to have a normal supply of this element. Leader samples from the former gave 0.5 to 0.8 per cent. MgO for the lower leaves, while the Waimea West leaders stayed healthy in the following season with the much lower magnesium content of 0.3 per cent. MgO. The Stoke samples may have been abnormally high in magnesium owing to an inverse relationship between the magnesium and potassium intake, or, on the other hand, at the Waimea orchard, where no magnesium had ever been applied on a soil of naturally low magnesium content, the trees may have been dangerously low in this element, with the possibility of deficiency symptoms appearing at a later period.

Nor is it possible from the figures given in the preceding tables to generalize on the distribution of these elements with position on the leader. Thus magnesium showed a rise towards the top of the shoot in the Tasman samples but no such rise until later in the season in the Stoke samples. Similarly, potassium was highest in the top leaves at Stoke but not in the other samples. The distribution of potassium or magnesium in the leader appeared to be dependent on the available supply of both these elements to the plant.

Results for magnesium-deficient leader leaves do show that the top leaves are not suitable for the diagnosis of this deficiency or for tracing the effect of magnesium treatment on the magnesium status of the plant. From Table I it will be seen that on the leader shoot the leaves of the lower half give the best indication of a shortage of magnesium. This is confirmed by further detailed work to be published later.

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## THE DETERMINATION OF MOLYBDENUM IN SOILS

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### Summary

A new procedure for the estimation of traces of molybdenum in soils is described. After ashing, the sample is disintegrated with hydrofluoric acid and dissolved in hydrochloric acid. Ether extraction of the solution separates molybdenum from interfering elements present in soils, with the possible exception of rhenium. In particular, interferences from titanium and chromium are avoided. The addition of citric acid reduces the amount of vanadium extracted to a sufficiently low level. Molybdenum is estimated finally as the thiocyanate complex.

A COMPLETE investigation of the role of molybdenum in animal nutrition requires a study of soil, plant, and animal relationships. Before such a study can be undertaken, reliable methods for the estimation of molybdenum in these materials must be available. An account has already been given(3) of an improved procedure for the determination of small amounts of molybdenum in plant and animal materials. The present work extends this research to include soils.

Since the average molybdenum content of soils appears to be about 0.5 p.p.m., it is necessary to use a sample of at least 5 g. to provide a sufficient concentration of molybdenum in the final solution for reliable colorimetric estimation.

Several methods have been proposed. That due to Stanfield(6) consists of prolonged acid extraction of a 200 g. sample on a steam bath, followed by direct precipitation of molybdenum as the sulphide and colorimetric estimation as the molybdenum thiocyanate complex. Such a procedure has several disadvantages; a very large sample is required, several days' continued extraction on a steam bath is necessary, and direct precipitation of molybdenum as the sulphide is difficult when only a minute quantity of molybdenum is present, as the precipitate tends to become colloidal. Sandell's method(4), which was originally proposed for the analysis of silicate rocks, consists of fusion with sodium carbonate, acidification of the aqueous extract of the melt, and colorimetric estimation of molybdenum as the thiocyanate. With a 5 g. sample, however, fusion is difficult and introduces a considerable quantity of salt.

In this laboratory, when Sandell's technique was applied to soils, it was found that an extraneous yellow colour, which was extracted by amyl alcohol, developed. Wet-digestion of the soil sample with sulphuric, perchloric, and nitric acids led to an interfering colour which was also found occasionally when soil-contaminated pasture samples were analysed for molybdenum. Absorption spectra measurements suggested that the interference was due to titanium, and it was found that sufficient titanium was present in the solutions concerned to give a false colour. Similarly, working with New Zealand soils, Watson(9) found that an ether-soluble yellow compound was produced, presumably due, also, to titanium.

Using the colorimetric procedure described earlier(3), it was found that 0.7 mg. of titanium is sufficient to cause significant interference. For a 5 g. sample this corresponds to 0.03 per cent. of titanium dioxide. Soils are unlikely to contain less than 0.5 per cent.(7), and in some New Zealand soils the figure may exceed 10 per cent.(2). As a method for the determination of a trace element should not be affected by other constituents of any sample, it has been necessary to consider all possible sources of

interference. Levels at which foreign substances cause an error exceeding  $0.3 \mu\text{g.}$  of molybdenum have already been determined(3). These levels are expressed below in two forms; the second column gives the number of milligrams, and the third column expresses these results as percentages for a 5 g. sample.

INTERFERENCE LEVELS

Ion.	Mg.	Percentage.	Ion.	Mg.	Percentage.
Ag+ ..	0.1	0.002	Al <sub>2</sub> O <sub>3</sub> ..	1.5	0.03
WO <sub>3</sub> ..	0.13	0.003	Cu++..	5	0.1
V <sub>2</sub> O <sub>5</sub> ..	0.7	0.01	Pb++..	10	0.2
TiO <sub>2</sub> ..	1.2	0.02	Co++ ..	12	0.2
Cr <sub>2</sub> O <sub>3</sub> ..	1.5	0.03	UO <sub>3</sub> ..	12	0.2
Hg++ ..	1.5	0.03	F- ..	20	0.4

Figures for rhenium, rhodium, and platinum are not known.

Low results which were obtained in the presence of aluminium using the wet-digestion technique(3) were found to be due to adsorption of molybdenum on the aluminium hydroxide precipitate when the solution was made alkaline with ammonia. Provided aluminium is not precipitated, no error is introduced. Of the remaining substances listed, titanium, chromium, vanadium, and possibly tungsten must be expected in soils in concentrations greater than the wet-digestion method allows. Because of the presence of interfering substances it was necessary either to free the solution from the elements concerned or to isolate molybdenum from the solution.

Preliminary experiments showed that precipitation of titanium and chromium as hydroxides was not a satisfactory practical method of freeing molybdenum from these elements, due to adsorption of molybdenum on co-precipitated hydroxides of iron and aluminium normally present. Consequently, isolation of molybdenum from the other metals present in solution was necessary.

Direct precipitation of molybdenum as the sulphide is difficult with small amounts. However, von Hevesy and Hobbie(8) have reported that copper sulphide acts as a collector for molybdenum. This was confirmed for the low level studied here, but, as possible interference in the final estimation limits the amount of copper that may be present to approximately 5 mg., difficulty was experienced in obtaining quantitative recoveries. Of the other metals which have been tried as collectors, cadmium appeared to be the most suitable, provided a small amount of copper was also present to give a sufficiently coarse precipitate to filter easily. However, small quantities of interfering elements were retained tenaciously by the precipitate even after prolonged washing, so that this method was not generally applicable to soils.

An alternative method of isolating molybdenum from most metals is by ether extraction of its solution in aqueous hydrochloric acid(5). Until now, use does not appear to have been made of this method. By this means a good separation may be obtained from all interfering substances except tungsten, vanadium, and, perhaps, rhenium. Unfortunately, as no rhenium salts were available, its behaviour towards ether extraction could not be tested. Ferric iron is quantitatively extracted, but ferrous iron remains in the aqueous layer. However, it is not practicable to use a reducing agent to convert all the iron to the ferrous state, since under these conditions molybdenum cannot be extracted completely.

An investigation of the effect of acid concentration on the amount of molybdenum extracted showed this factor to be an important one. The partition coefficient increases rapidly with the strength of the hydrochloric acid until the solution is approximately 6*N*—i.e., sp. gr. 1.10—and decreases rapidly as the acid concentration is further increased. From a solution in 6*N* hydrochloric acid, approximately 70 per cent. of the molybdenum is extracted on shaking with an equal volume of ethyl ether, so that under these conditions more than 99 per cent. of the molybdenum is removed using four extractions. The molybdenum is recovered quantitatively by washing the combined ether extracts with water. The concentration of tungsten in the final solution is sufficiently reduced by removal of tungstic acid when the acid solution is filtered and by the relatively unfavourable partition coefficient of tungstic acid. Molybdenum is not retained by the precipitate. By adding citric acid before treatment with ether, the extraction of vanadium is decreased to a level at which interference is avoided.

Using the ether extraction technique, the presence of much salt is undesirable, owing to clogging of the stopcocks in the separating funnels, and for this reason sodium carbonate fusion is impracticable. Treatment of the sample for several hours with boiling hydrochloric acid was found to leave appreciable quantities of molybdenum in the insoluble residue, particularly with pumice and clay soils. Evaporation with hydrofluoric and sulphuric acids gives a residue almost completely soluble in hydrochloric acid, and this technique is used in the present method.

#### METHOD

From 10 to 20 g. of soil dried at 100° c. and ground to pass a screen  $\frac{1}{4}$  in. mesh are weighed into a silica basin and ashed at 400–450° c. for three hours. At higher temperatures, molybdenum may be lost by volatilization as the trioxide(1). For peats, larger samples and longer periods of ashing are necessary. Approximately 5 g. of ash are weighed in a platinum basin, and 10 ml. of distilled water, 2 ml. of concentrated sulphuric acid, and 10 ml. of 50 per cent. hydrofluoric acid are added. The basin is heated gently on an electric hot-plate until most of the hydrofluoric acid and water has evaporated, and then more strongly to remove the residual sulphuric acid. The residue is taken up in hydrochloric acid (sp. gr. 1.10) and transferred to a 100 ml. beaker. The solution is evaporated to approximately 5 ml., filtered through a Whatman No. 40 filter paper (5.5 cm.) into a separating funnel, and the residue is washed thoroughly with more hydrochloric acid until the volume of filtrate is approximately 20 ml. After adding 4 ml. of 40 per cent. citric acid in constant boiling-point hydrochloric acid, the solution is extracted with four 25 ml. portions of ether. The combined ether extracts are shaken with three 10 ml. lots of water, the aqueous extracts being run into an 8 in.  $\times$  1 in. Pyrex test-tube containing three small glass beads. This aqueous solution contains all the molybdenum and most of the iron originally present in the soil sample. After evaporating the solution to about 3 ml., 4 ml. of constant boiling-point hydrochloric acid is added. Two milligrams of ferrous iron are added to the blank and any standards which may be included. Three millilitres of 10 per cent. potassium thiocyanate solution are then added to each sample, and sufficient 40 per cent. stannous chloride in 1 : 9 hydrochloric acid to destroy the red colour due to iron is run in from a coarse burette. The approximate volume required is noted, since a slight correction has to be applied. The volume is adjusted to 25 ml. with distilled water, and molybdenum is then estimated as described in an earlier paper(3).

If much iron is present, the stannic oxide produced occasionally has a stabilizing action when amyl alcohol is shaken with the solution, and a water-in-alcohol emulsion may be produced. In this case the emulsion is transferred to a centrifuge tube and centrifuged for ten minutes at 2,000 r.p.m., after which the clear amyl alcohol layer may be filtered off in the usual manner. Recoveries showed that there was no absorption of molybdenum on the precipitate.

### DISCUSSION

The previous paper showed certain substances to interfere seriously in the determination, whereas other workers have stated that no such interference is encountered. This discrepancy is to be attributed to the level of molybdenum studied. Interference equivalent to, say 0.3  $\mu$ g. of molybdenum is negligible in a sample containing 1 mg. of molybdenum, but is obviously important if only a few micrograms are present.

To establish that the proposed method of analysis would be generally applicable to such variable material as soil it was necessary to show that the concentration in soils of elements that interfere with the colorimetric estimation of molybdenum is not high enough to make the results unreliable. To standard solutions containing 10  $\mu$ g. of molybdenum varying amounts of interfering substances were added. These standards were then tested as described in the method for soils. It was found that less interference than is equivalent to 0.3  $\mu$ g. of molybdenum was caused by the presence of—

0.5 g. $\text{TiO}_2$	0.25 g. $\text{Cr}_2\text{O}_3$
25 mg. $\text{V}_2\text{O}_5$	5 mg. $\text{WO}_3$
5 mg. $\text{Ag}^+$	

These levels are believed to exceed those likely to be met with in a 5 g. sample of any soil ash. It was necessary also to investigate whether, in a strongly acid solution of phosphate, molybdenum would be as readily extracted into ether, or whether phosphomolybdic acid would be formed, leading to significantly lower results. Standard solutions containing phosphate ion equivalent to 0.25 g. of phosphorus pentoxide were treated as above and quantitative recoveries were obtained. This level appears to be the maximum which may be met with in any sample. As rhenium is believed to be very rare, no substances are likely to be present in sufficient amounts to cause interference.

As a test of the present method a series of recovery experiments was carried out in which known amounts of molybdenum were added to soil samples before acid treatment. The results obtained are given in Table I and show that quantitative recoveries are obtainable by this method.

TABLE I.—RECOVERY OF MOLYBDENUM ADDED TO SOIL

Description of Ash (5 g. of each Sample used).	Percentage Ash.	Mo added, (micro- grams.)	Mo found.	Mo calculated.	p.p.m. Mo in Soil.
Clay, Waikiekie..	97.2	..	2.2	..	0.43
..	..	10.0	11.7	12.2	..
Ngaio silt loam, G33	90.3	..	2.8	..	0.50
..	..	5.0	7.7	7.8	..
Loam, E982 ..	72.2	..	4.4	..	0.63
..	..	5.0	9.4	9.4	..
Peat, F1463 ..	11.3	..	5.0	..	0.28
..	..	10.0	14.8	15.0	..
Argillaceous sand, G455 ..	98.9	..	6.0	..	1.19
..	..	10.0	16.1	16.0	..
Pumice, Thornton, E1602	96.5	..	6.6	..	1.28
..	..	5.0	11.0	11.6	..
Pumice, Wairoa..	92.9	..	6.5	..	1.21
Clay, Wallaceville ..	94.0	..	2.2	..	0.41

A detailed investigation of the factors involved in the development and stability of the molybdenum thiocyanate colour has already been made(3), and the results obtained there have been used as the basis of the final estimation in the present method. One significant point of difference, however, is in the salt concentrations of the final solutions in the two methods. It was shown previously that salt concentration had a slight effect on the intensity of colour of the iso-amyl alcohol extract, and in the earlier method this effect was sensibly constant because the salt concentration was large and varied only slightly. In the present method iron is the only major soil constituent which is appreciably extracted, and an approximate indication of the amount present is obtained by rough titration with 40 per cent. stannous chloride (1.8 per 0.5 g.  $\text{Fe}_2\text{O}_3$ —i.e., per 10 per cent.  $\text{Fe}_2\text{O}_3$  for a 5 g. sample). The presence of large amounts of iron causes a slight decrease of the colour obtained for a given amount of molybdenum. The approximate error is as follows:—

Grams $\text{Fe}_2\text{O}_3$ in sample..	0.5	1.0	1.5	2.0	3.0	4.0
Percentage decrease in colour	1	2	3	4	7	10

Over the range for normal soils the correction required is small and for most purposes could be neglected. The effect observed with iron in a sample includes that due to variation in the amount of stannous chloride and the associated change in concentration of hydrochloric acid. The correction factor, which is probably due to change of concentration of acid, increases more rapidly as iron content increases and the magnitude of the correction may be predicted from Fig. 1 of the previous paper(3).

The present technique is also applicable to the estimation of molybdenum in soil-contaminated pasture samples. If a pasture sample contains an appreciable quantity of soil, this is clearly shown by the appearance of the digest remaining at the close of the wet digestion, which under these conditions includes a grey or brown residue. After neutralization with ammonia and evaporation almost to dryness as described in the wet-digestion technique, the sample is taken up in constant boiling-point hydrochloric acid and treated in the same manner as a soil extract. This procedure was found to give quantitative recoveries when standard molybdenum solutions were tried.

The preparation of a calibration curve showed that for any given quantity of molybdenum the intensity of the colour of the amyl alcohol layer was approximately 3 per cent. greater using the present method than it was when the wet-digestion technique was employed. This is in agreement with previous observations(3) and is due to the marked difference in salt concentration in the two methods.

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## THE CHARACTERISTICS OF THE MILK-EJECTION CURVE OF NORMAL DAIRY COWS UNDER STANDARD MILKING CONDITIONS

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### Summary

By the use of a milk-flow-recording apparatus the volume/time curves for twelve cows milked under strict machine-milking conditions without hand stripping have been traced throughout most of a lactation period.

From these curves the following figures have been obtained: total milk weight, actual milking-time, average milk-flow rate, maximum milk-flow rate, weight of machine strippings, and time taken to start milking.

A "sight glass" has been developed which indicates when the milk flow falls to a predetermined level.

THE present paper describes the results obtained by recording the milk-ejection curves of a dozen cows at intervals throughout the season. The apparatus used was similar to that described in an earlier paper(1), with the modification that two main cylinders were used on the measuring unit, one of which could be cut out by turning a tap. By this means the recorder could be operated with two scales so that when low milk yields were being considered the scale could be expanded.

The main object of the work was to ascertain quantitatively what happens when cows are milked mechanically under strict non-hand-stripping conditions. The conditions of the experiment were as follows:—

*Milking-machine.*—Simple single-bail orthodox releaser plant with slide pulsators and 7 in.  $\times$  6½ in. rotary vacuum pump.

*Teat-cups.*—Telescopic type fitted with Standard ¾ in. straight inflations, which were tightened regularly by the use of the telescopic feature. This enabled a constant tension to be maintained throughout the life of the inflations, which were changed every six weeks.

*Pulsator Rate.*—42.

*Pulsator Ratio.*—50 : 50.

*Pulsator Performance.*—Satisfactory(8).

*Vacuum.*—14½ in., maintained accurately by oil-damped, weighted relief valve.

*Shed Technique.*—The cows were leg-roped. The udders were washed immediately prior to the teat-cups being applied. Except during a short preliminary period when "machine stripping" was omitted, the teat-cups, as soon as the milk flow declined as indicated by the "sight glass" on the machine, were pulled down by hand ("machine stripping"), and when milk flow had again dropped the cups were removed and the cows let out. The cows were milked in a regular order and milking started at the same times each day.

Details of the cows used in the experiment and their previous season's treatment are set out in Table I.

At the beginning of the experiment records were made twice a week, but during the main period weekly records only were made. The recording unit was used in such a way that its operator was not in evidence throughout milking, thus ensuring normal conditions for the tests. When the flow rate had fallen to about 0.5 lb. of milk per minute according to the graph in the recorder, the signal was given by an indicator lamp for the cups to be pulled down. When the rate had fallen to this level again, the cups were removed.

TABLE I

Cow No.	Age.	Productions.*				Milk Conditions, 1943-44	Calving Date.	October Test.		Milk Conditions, 1944-45†.
		1940-41.	1941-42.	1942-43.	1943-44.			Milk.	Fat.	
O4	5	..	?	301/286	293/286	N.S.; cup manipulation ..	1/10/44	44	44	Complete N.S.
X4	6	305/272	270/298	297/263	350/277	" ..	20/8/44	28	34	" N.S.; does not
277	5	..	162/224	216/206	Abort.	" ..	13/8/44	34	37	milk out in R.H.
34	4	..	..	213/238	231/207	Hand stripped ..	27/9/44	31	39	Hand strip.
58	4	..	..	271/270	276/232	N.S.; cup manipulation ..	25/7/44	24	33	N.S.; cup manipulation
83	3	Calved 14/2/43				Hand strip ..	23/9/44	25	32+	irregular.
113	2	..	..	..	118/193	" ..	20/9/44	24	35	Hand strip.
121	2	..	..	..	..	" ..	10/10/44	..	..	Complete N.S.
142	2	..	..	..	..	" ..	25/9/44	23	35	" "
180	2	..	..	..	..	" ..	15/9/44	26	41	" "
126	..	..	..	..	..	" ..	30/6/44	26	34	" "
128	..	..	..	..	..	" ..	30/8/44	36	41	" "

\* Pounds of butterfat/days in milk.

† These were the conditions prior to starting the experiment using the recording apparatus.

NOTE.—Cup manipulation: weight applied to cups at cessation of milk flow. Complete N.S.: cups removed at cessation of milk flow.



An indicating device was developed subsequently (see Appendix) which indicated the fall in milk flow quantitatively. This was used between recordings to ensure uniformity of milking conditions.

At the end of the season the recorder was used with the single measuring cylinder in operation, thus expanding the scale and increasing the accuracy.

In order to simplify the making of comparisons and in order to put some aspects of the results on a quantitative basis, certain characteristics of the milk-flow curves have been measured. The following are the characteristics:—

Total milk weight (W). (NOTE.—As this is measured by the fifty studs in the measuring cylinder of the recorder, high percentage error may occur at low values. Each stud represents 0.544 lb. of milk when the two main cylinders are used and 0.281 lb. when one cylinder is used.)

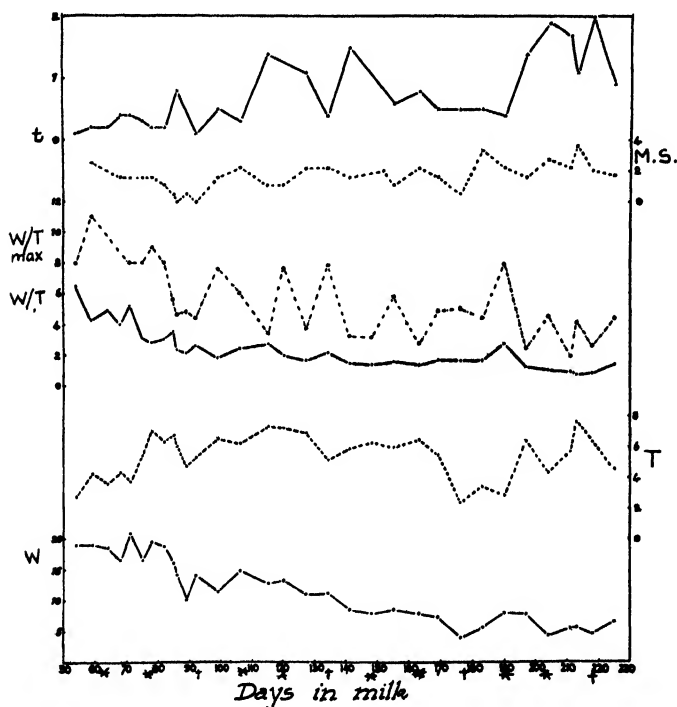


FIG. 1.

Total milking-time (T). This is the time from the application of the teat-cups until the last unit of milk is recorded. Only a small error is involved in this measurement.

Average milk-flow rate (W/T). To speed up the determination of this quantity a simple protractor was used, as for the next figure (W/T max.). This involved an error under certain conditions, which explains why calculated values of W/T do not always agree with the values given. However, the variance of the results is such that their significance depends on the numbers and not on the accuracy of a single determination.

Maximum milk-flow rate (W/T max.). The reason for taking out this figure was primarily to have some indication of the liquid-handling requirement of the milking-machine.

Machine strippings (MS). This figure is the amount of milk given after the teat-cups had been pulled down. As the amounts are small, in general the figure must be treated as an index of the tendency towards giving strippings rather than an exact measure.

Time to deliver first unit of milk (0.544 lb.) ( $t$ ). This figure gives an accurate indication of the time taken for the cow to deliver a constant small amount of milk. It is therefore an index of the time taken after the application of the teat-cups for the milk to start flowing.

The units used in measurement are: W lb., T minutes,  $t$  minutes.

The asterisks indicate the days on which teat-cup liners (inflations) of the milking-machine were tightened, and the daggers the days on which they were changed.

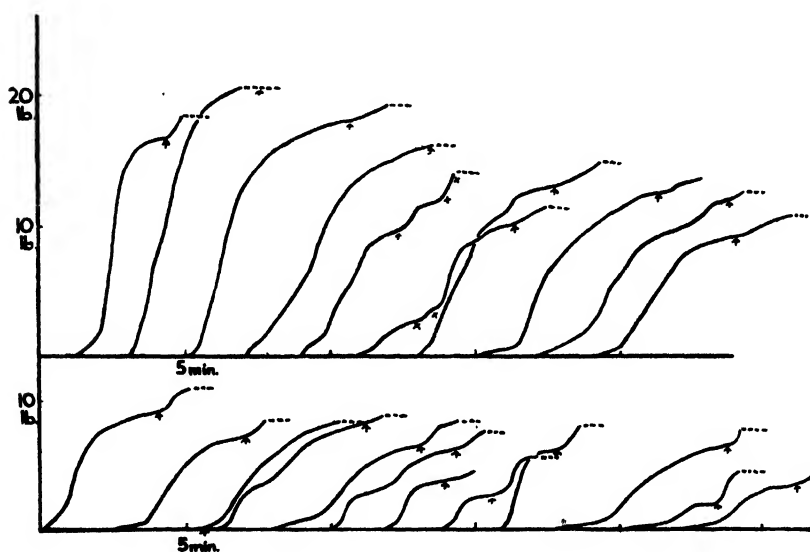


FIG. 2.

The data obtained during the experiment are set out as a series of milk-flow curves obtained as tracings from the original recordings and as a series of graphs. Each of the twelve cows will be discussed in order of milking and a summary of the chief characteristics of the group given.

*Cow 1 (04).*—Figure 1 sets out the variations in the characteristics of the milk-flow curves throughout the season, while Fig. 2 gives the tracings of the actual milk-flow curves. The decline in milk-flow rate with declining yield is one of the features of this cow. There is no great tendency for machine stripping to increase. The time taken to start milking tends to increase and become erratic.

In the milk-flow graphs (Fig. 2) the point marked with an arrow shows where the cups were pulled down. The dotted portion is the continuation of the curve after the last unit of milk has been delivered. A small x indicates a point at which the cows had kicked the teat-cups off. The ideal curve would have no "foot" and would stop suddenly when the gradient starts to decrease. The second curve for cow 1 shows these characteristics. Generally, however, the curve departs considerably from the ideal.

*Cow 2 (x4).*—Figure 3 sets out the changing characteristics and Fig. 4 the actual milk-flow curves. Again there is a fall in average milking-rate as the yield declines. Machine strippings do not increase, but there is a marked

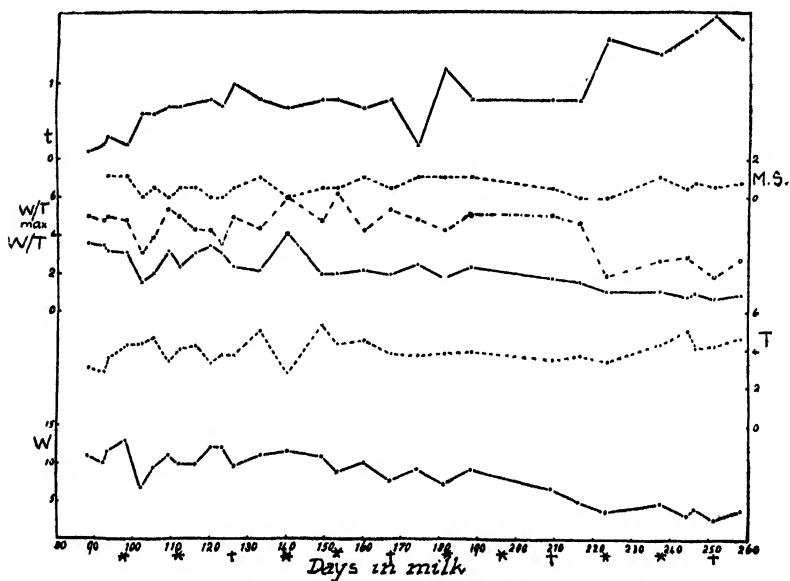


FIG. 3.

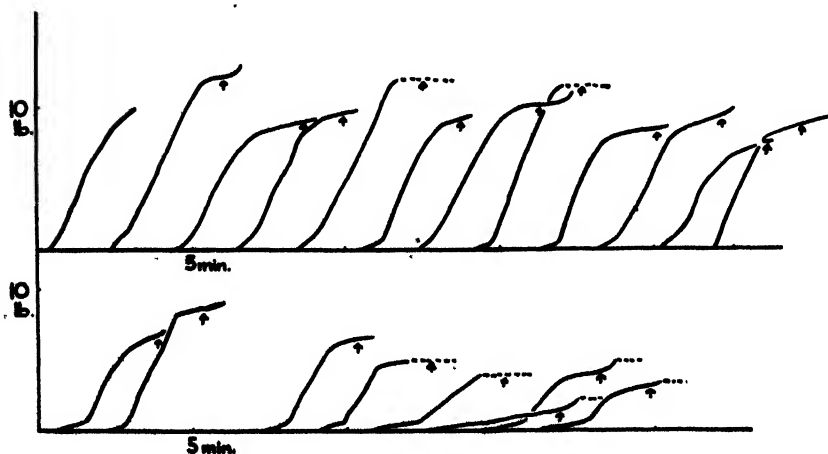


FIG. 4.

rise in starting-time ( $t$ ). The milk-flow curves are more regular in this case, though towards the end an irregular shape develops. Until the end of the season the curve shows a low starting-time and a good average flow rate.

*Cow 3 (58).*—Figures 5 and 6 set out the results for this cow. Average milking-rate again declines, machine strippings do not vary significantly, and starting-time shows no great variation except for the last point on the

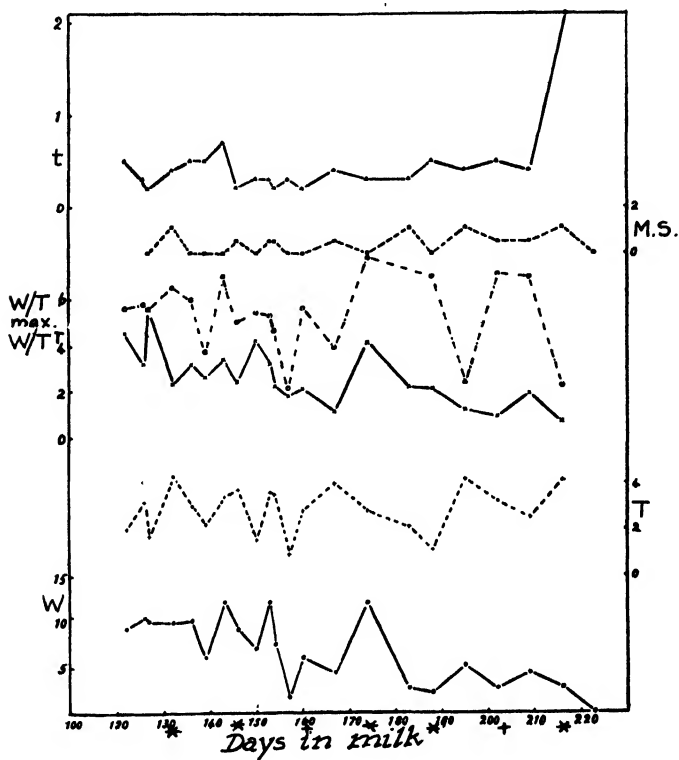


FIG. 5.

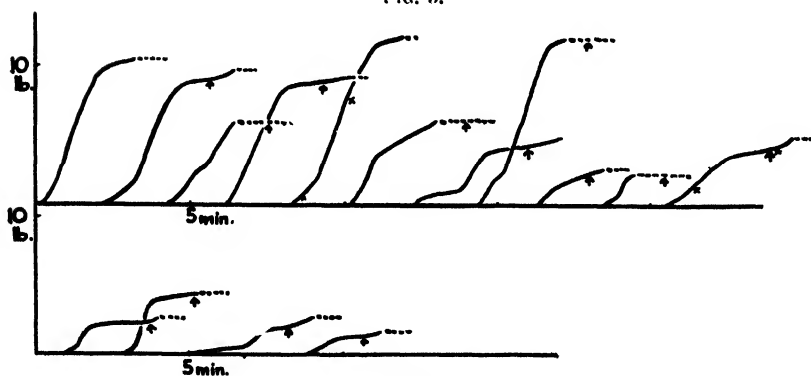


FIG. 6.

graph (Fig. 5), which is very high. Figure 6 shows this cow to be somewhat irregular in yield. The last curve shown followed a recording at which no milk was given. Data for this curve are not given in Fig. 5. This cow calved earlier than the previous ones and the first part of the season was missed.

*Cow 4 (126).*—From Figs. 7 and 8 it will be evident that we have again a decline in milking-rate and no significant change in machine strippings. The starting-time shows a trend upwards, and generally the results are fairly

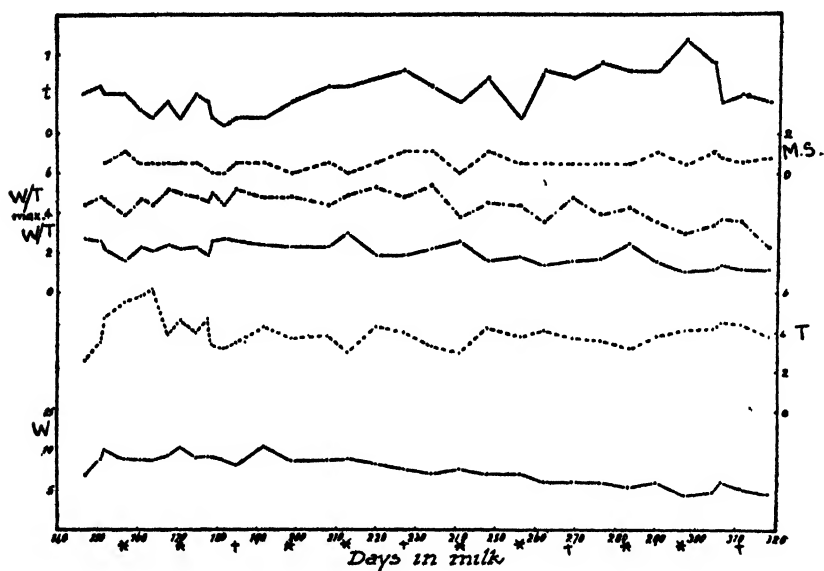


FIG. 7.

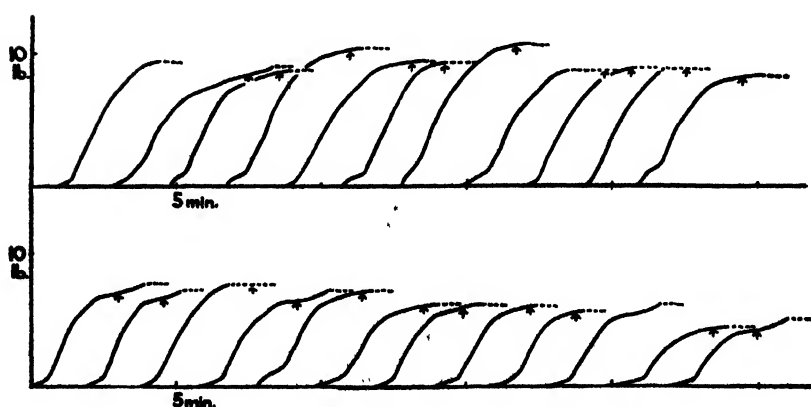


FIG. 8.

regular. Figure 8 shows that the milk-flow curves maintain a constant characteristic throughout the season. This cow has excellent machine-milking characteristics.

*Cow 5 (83).*—Figures 9 and 10 show this cow to be a thoroughly unsatisfactory milker. She has a low average milking-rate, a variable and sometimes long starting-time, and an erratic yield. She dried off

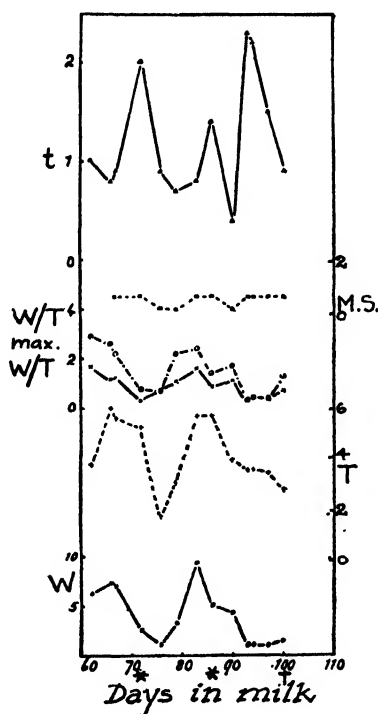


FIG. 9.

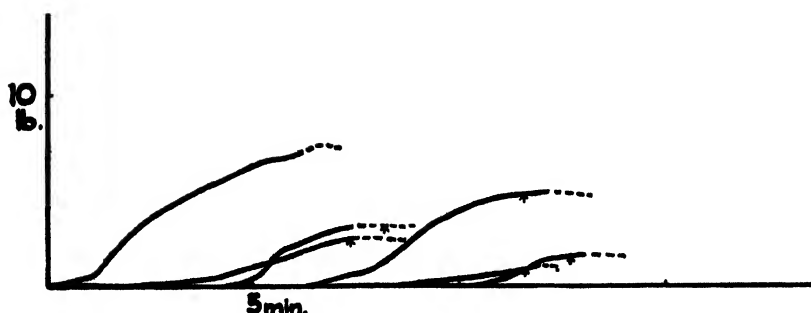


FIG. 10.

prematurely under the milking conditions to which she was subjected. It is interesting to note that she does not give much milk as machine strippings. Such a cow is obviously unsuited to machine milking. During the previous season she milked for 193 days when hand stripped.

*Cow 6 (180).*—The characteristics of this cow are shown by Figs. 11 and 12. Again there is a falling rate curve and a slight rising tendency on

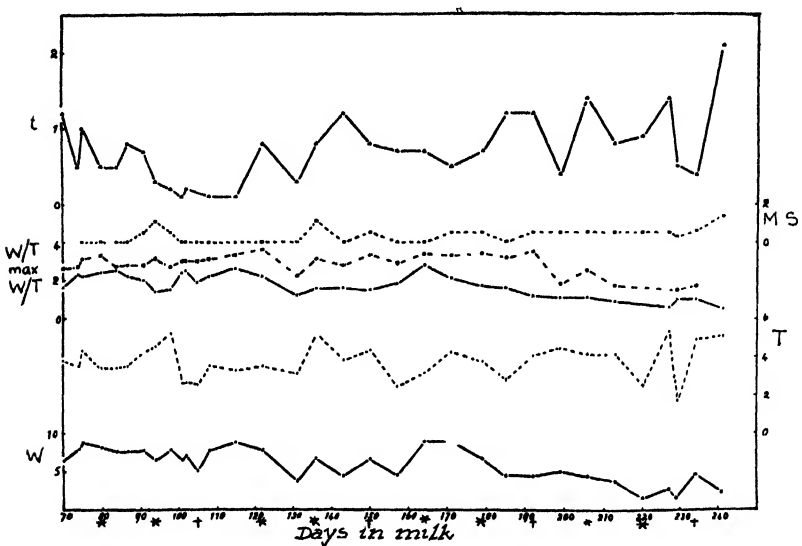


FIG. 11.

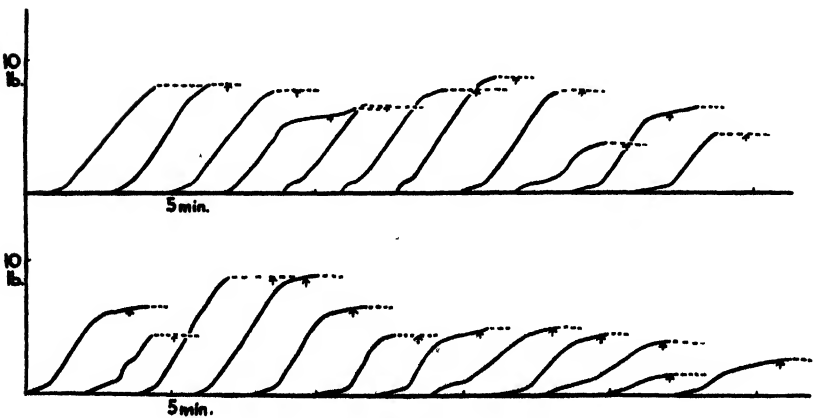


FIG. 12.

the part of the starting-time. Machine strippings, which are a small quantity, show a tendency to rise. Generally the milk-flow curves are fairly regular. The cow is a good machine milker.

*Cow 7 (121).*—Figures 13 and 14 show this cow to be an excellent machine milker. Machine strippings are negligible, milking-time is low, there is no significant rise in starting-time, and the average flow rate is very regular.

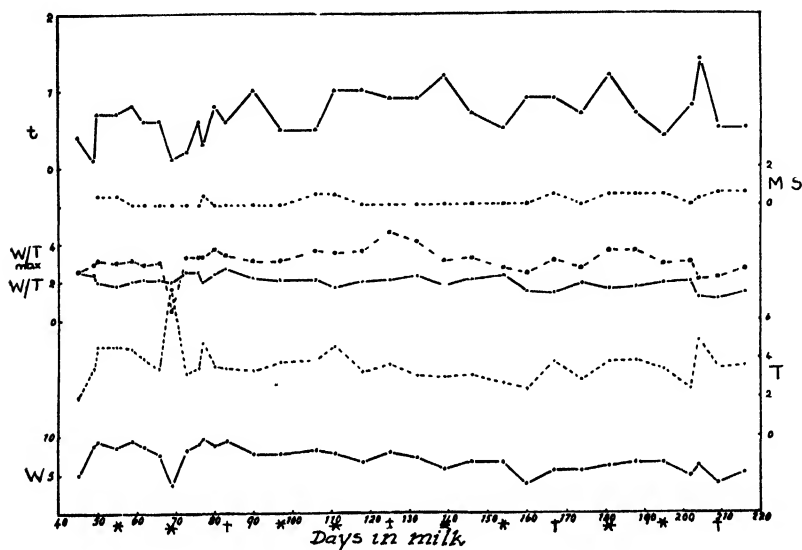


FIG. 13.

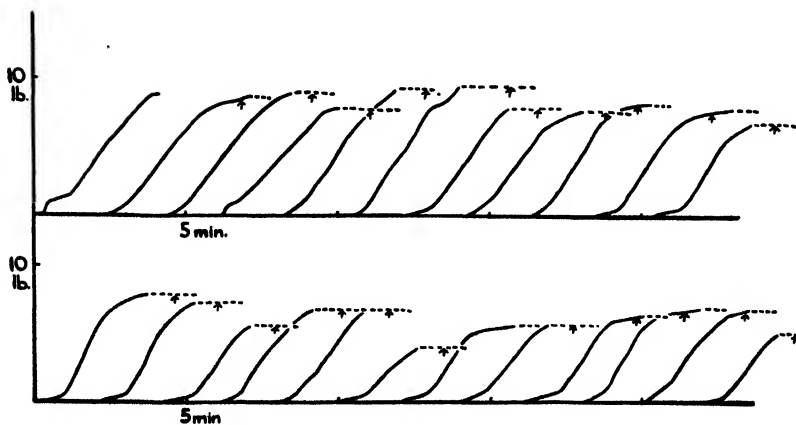


FIG. 14.



*Cow 8 (113).*—This cow was followed over a period nearer to the end of lactation than the preceding case. Again (Figs. 15 and 16) we have good regular characteristics until, as the end of lactation approaches, the

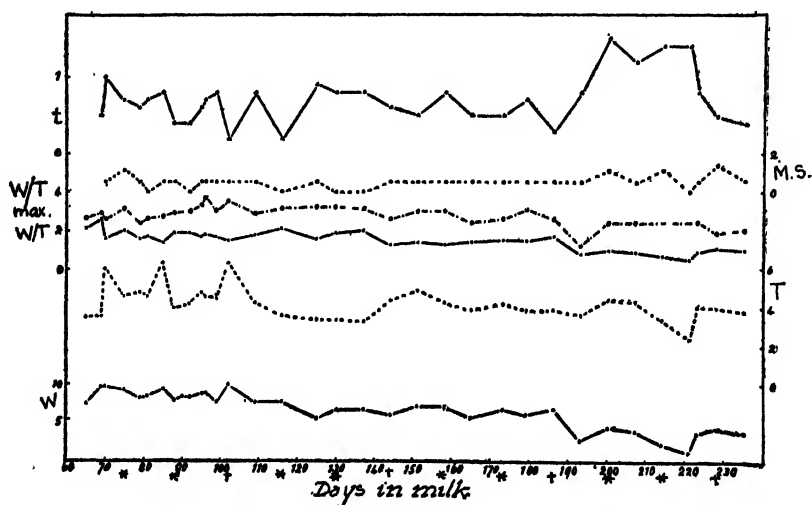


FIG. 15.

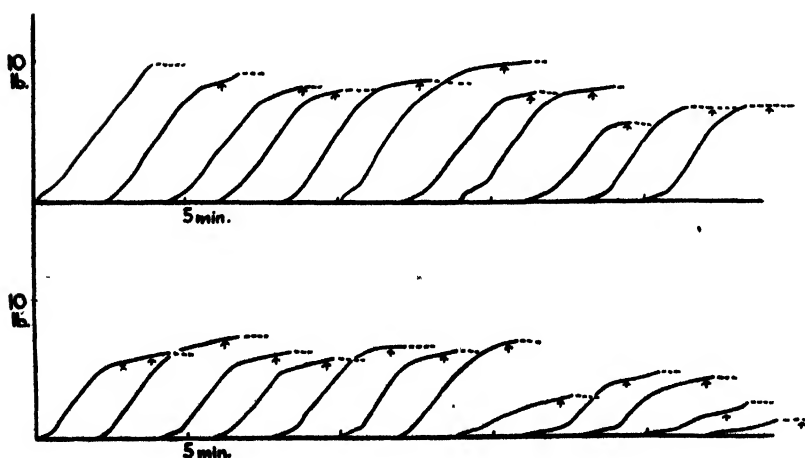


FIG. 16.

starting-time becomes irregular. There is a steady fall in average milk-flow rate. Machine strippings are low, but show a variable increase towards the end of the period. The milk-flow curves (Fig. 16) are very regular until the end of lactation becomes evident.

*Cow 9 (277).*—Figures 17 and 18 again give a picture of falling milking-rate and increasing starting-time. In this case the starting-time shows a very marked rise at the end of the curve. Machine strippings show a slight

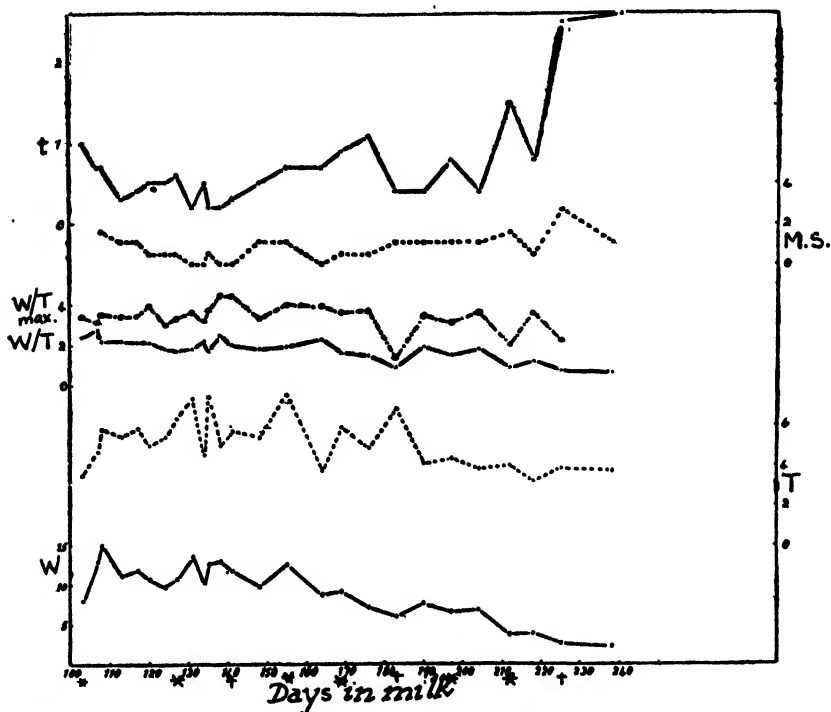


FIG. 17.

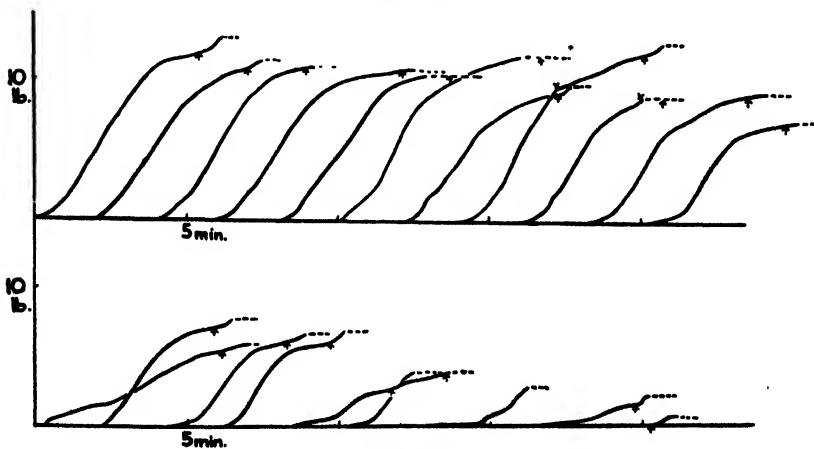


FIG. 18.

rising tendency towards the end. The milk-flow curves are of good form until the latter weeks of the season, when they become erratic and show poor characteristics. The "letting down" of the milk has become delayed and irregular.

*Cow 10 (128).*—The results set out in Figs. 19 and 20 show an unusual amount of variation in the amount of machine stripping during the latter part of the period. Similarly, a marked variation in starting-time is to be seen. Average milking-rate shows a steady decline, while there is a distinct

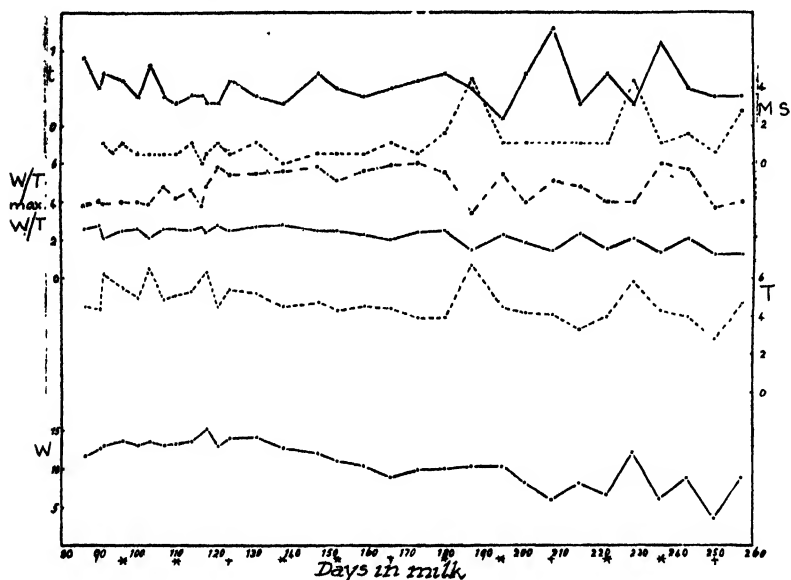


FIG. 19.

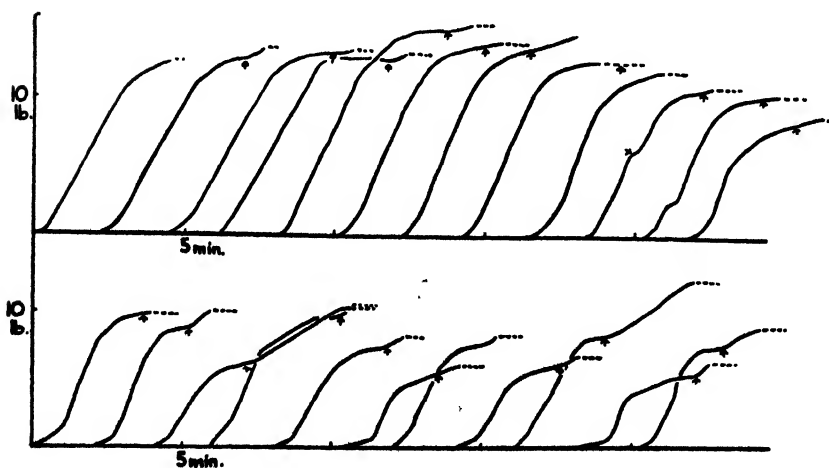


FIG. 20.

increase in the variability of yield towards the end. The milk-flow curves during the first parts of the period of test are excellent. Towards the end the curves show a remarkable increase in machine strippings on some occasions. This phenomenon shows no regularity.

*Cow 11 (34).*—Figures 21 and 22 show another picture of a cow unsuited to machine milking, though whether better results could be obtained by hand milking or by machine milking and hand stripping is open to doubt. The

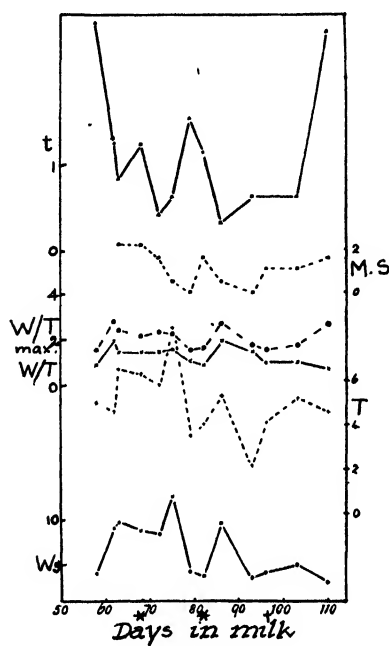


FIG. 21.

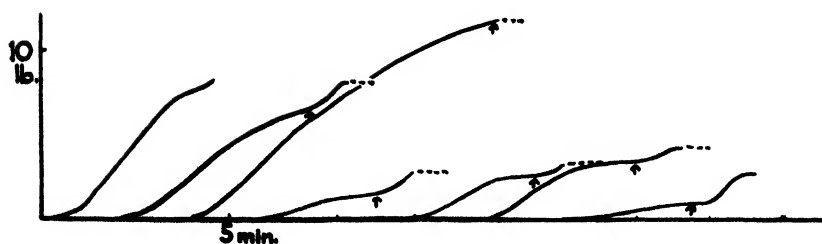


FIG. 22.

cow is a poor producer with erratic characteristics. The starting-time is very variable, as is the yield. The cow dried off prematurely. Though machine strippings are not great, this cow shows a relatively high percentage of strippings because of her poor yield.

*Cow 12 (142).*—The most striking feature of the characteristics of this cow as shown in Figs. 23 and 24 is the low average milking-rate. There is only a slight trend downwards in this figure, and it is followed closely by the maximum milk-flow curve, which in most cases is relatively independent

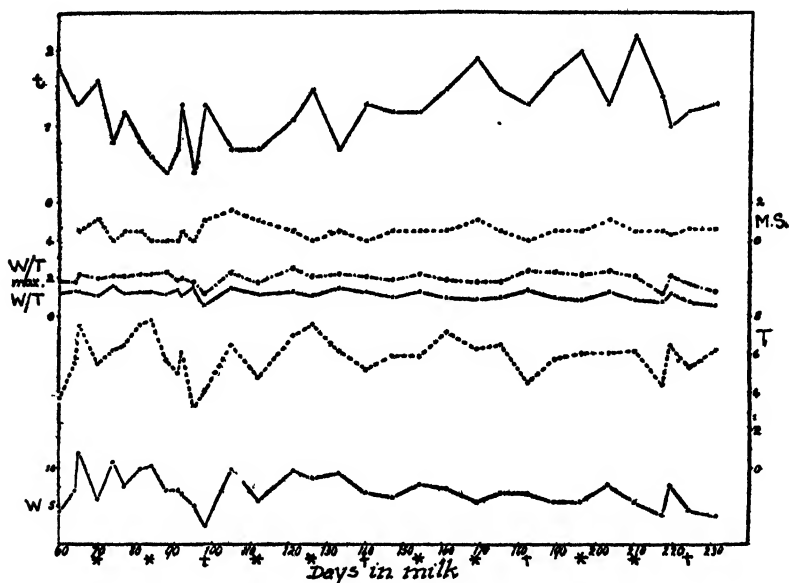


FIG. 23.

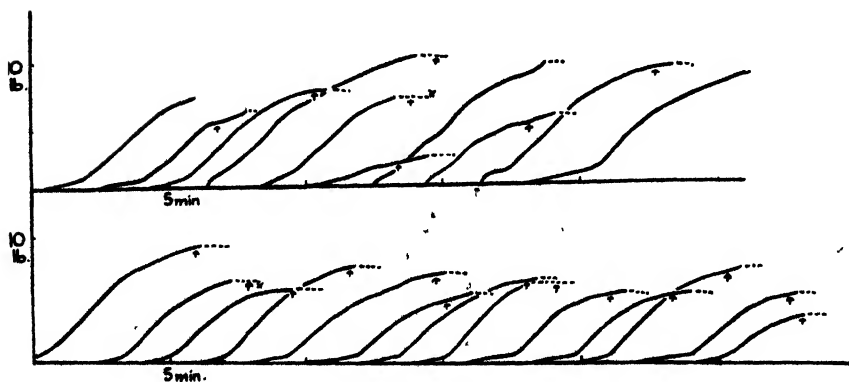


FIG. 24.

of the average figure. Considerable variation in the starting-time is to be seen, while machine strippings are steady and of low order. There appears to be no relation in this case between slow milking and machine strippings. The milk is probably let down normally, but is drawn slowly because of a small papillary duct or tight teat sphincter.

## DISCUSSION

The foregoing results are essentially a quantitative description of how a number of different sorts of cows milk under strict machine-milking conditions. The following generalizations are of some interest.

The average milk-flow rate tends to decline towards the end of the season. This means that the time taken to milk does not in general decrease.

The "machine strippings" do not increase significantly with declining yield when expressed in actual amounts. The percentage of milk yielded as machine strippings increases, however. This fact fits Petersen's suggestion as to the nature of machine strippings(2) as usually obtained—*i.e.*, that they are due to the teat-cups crawling up the teats and causing the upper portion of the lumen of the teat to be closed off.

There is a tendency for the starting-time (*t*) to increase and become erratic towards the end of lactation. This is a factor in reducing the average milking-rate. If the milk let-down reflex is to be regarded as a "conditioned reflex"(3, 4), this irregularity would have to be explained as being due to a failure of the suggested hormonal mechanism(3, 5, 6). Constant "reinforcement" strengthens a conditioned reflex(7), so that there should be no tendency for the nervous reflex component of the milk let-down process to fail towards the end of lactation. On the other hand, a tendency towards partial failure of the posterior lobe of the pituitary gland to supply adequate amounts of the "let-down factor" to ensure complete milking might well be associated with other endocrine changes accompanying failing lactation.

Table II sets out the average values of W, T, W/T, W/T (max.), MS, and *t* for each cow.

TABLE II.—AVERAGE VALUES

Cow No.	W.	T.	W/T.	W/T. (max.).	MS.	
O4 ..	11.5	5.34	2.38	5.48	1.82	0.69
X4 ..	8.45	3.95	2.23	4.34	0.57	0.83
58 ..	7.0	2.8	2.6	5.3	0.4	0.45
126 ..	7.77	4.01	2.03	4.38	0.56	0.51
83 ..	3.9	4.1	0.9	1.5	0.4	1.21
180 ..	5.94	3.71	1.71	2.90	0.32	0.72
121 ..	7.04	3.70	1.97	3.11	0.19	0.69
113 ..	6.58	4.22	1.51	2.76	0.49	0.71
277 ..	9.20	5.11	1.77	3.36	0.80	0.74
128 ..	10.60	4.65	2.24	4.76	1.12	0.53
34 ..	5.8	5.0	1.1	2.0	1.1	1.12
142 ..	6.84	5.90	1.15	2.0	0.50	1.22
	7.55	4.37	1.80	3.49	0.69	0.79

The average time taken for the milk to be let down, (*t*)—*i.e.*, 0.79 minutes—suggests that the application of the teat-cups is the main stimulus initiating the reflex. Ely and Petersen(3) show that the hormone secreted by the pituitary gland takes forty-five seconds to take effect. Where a cow takes a shorter time on the average to let down her milk, it is likely that the preliminaries to milking are acting as the stimuli to the let-down reflex.

An interesting point in this connection is the fact that milk let down appears to be necessary before the cow starts to milk at any significant rate, despite the fact that there is milk in the milk cistern which could be withdrawn. Is the sudden rise in pressure associated with the letting-down of the milk necessary to initiate flow by helping the vacuum to overcome the initial resistance offered by the teat sphincter? This point will be examined in future experiments.

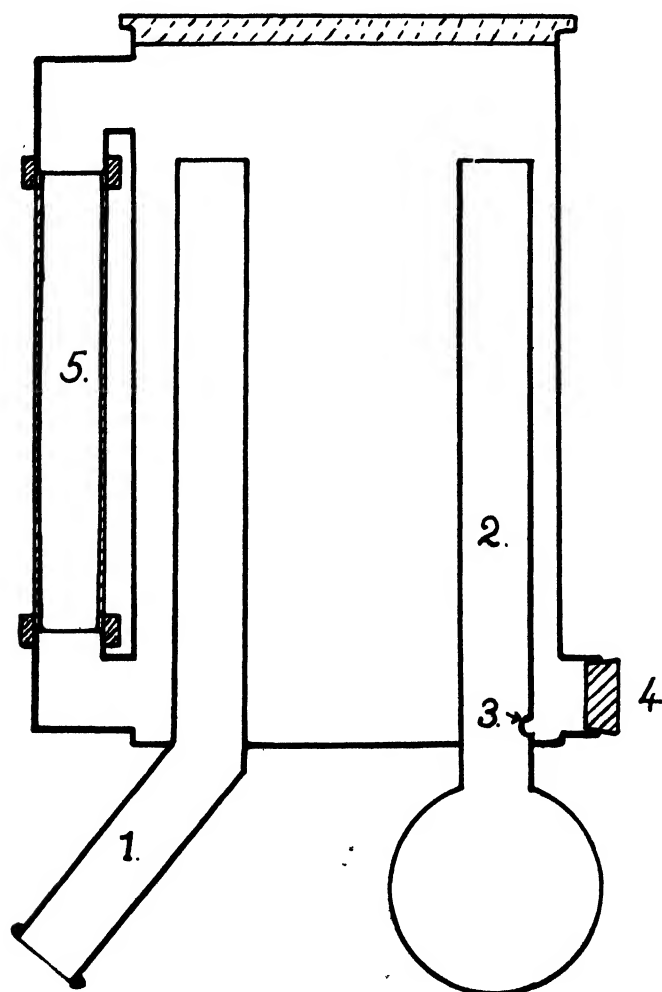


FIG. 25.

The average milking-time of 4.37 minutes is shorter than that obtaining on the average farm. In an investigation into non-stripping some time ago(8) it was found that the average rate of milking on the farms examined was 7.25 cows/hour/set of cups. This gives an average time of 8.3 minutes.

The time taken to change the cups should not exceed  $1\frac{1}{2}$  minutes, so that it is evident that, on the average, the cups are left longer on the cow than is necessary. A more precise means of determining when the cow has stopped milking would be a help in this connection. It is possible that the "sight glass" described in the Appendix to this paper may be developed into a suitable form for use on the ordinary farm.

In conclusion, it might be noted that under the conditions of milking described above, only one-quarter of the forty-eight showed signs of mild subclinical mastitis during the season. This would indicate that there is no factor in the strict machine-milking procedure which is harmful to the health of the udder.

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#### APPENDIX

##### A MILK-FLOW-INDICATING SIGHT GLASS

THE object of this sight glass is to give a definite indication of the point at which the milk flow from the cow falls to a level at which we can regard the milking process as complete. As can be seen from the shape of the milk-flow curve, there is no sudden termination of milk flow. However, there is a more or less rapid change in rate of flow towards the end of milking. We have selected a rate of flow of about 0.5 lb. of milk/minute as a suitable point at which to regard milking as substantially complete. When the flow rate falls to this level, the cups are pulled down. When the rate again falls, the cups are removed.

Figure 25 shows the essentials of this indicator in section. The milk enters by tube (1), leaving the unit by tube (2), which is connected to the milk-pipe of the machine. The level of the milk in the device is shown in tube (5), which is made of Pyrex glass. Just where tube (2) leaves the bottom of the unit a small hole is drilled. This hole determines the rate at which the milk flows from the device and is adjusted so that the milk level is half-way up tube (5) when the flow is 0.5 lb./minute. When the flow is above this level, tube (5) fills; when it is below, the tube empties. At (4) is a rubber bung which can be removed for the purpose of cleaning hole (3). So far this has been found unnecessary.

The device has been found very useful as a means of standardizing the point at which the teat-cups are removed. In a modified form it may be useful to the farmer.

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## THE INHERITANCE OF "PARROT-BEAK" IN NEW ZEALAND MILKING SHORTHORNS \*

By J. M. RANSTEAD, Matangi

[Received for publication, 12th June, 1946]

### Summary

Parrot-beak is inherited as a simple recessive autosomal lethal factor, and the condition is briefly described and illustrated.

The inheritance of parrot-beak in the author's strain of pedigree milking Shorthorns is traced back to the bull Hippo, whose immediate ancestors were imported from England.

It is suggested that the cases in the United States of America reported by Snyder, and by Heizer and Hervey, inherited the factor from the same source.

The possibility of unwittingly distributing lethal factors through bulls used at artificial insemination centres is stressed.

In 1935 Professor Snyder, of Ohio State University, reported the discovery of a recessive lethal factor in cattle which he named "parrot-beak" (1). In the heterozygous condition the calves are perfectly normal, and show no evidence of carrying the lethal factor. In the homozygous condition the factor produces calves with an abnormally shortened lower jaw, in which the molar teeth are impacted. These calves never live more than a few hours and may even be born dead.

This discovery was brought to the notice of Heizer and Hervey (2), who made an investigation of the milking Shorthorn herd in which the defect was found. There were not enough matings nor enough information to compute expected ratios of normal to defective offspring and to compare such ratios with those actually observed. In only five cases could the parentage be traced accurately, but altogether there had been about eight cases of this defect in the herd. The close relationship of the five calves whose parentage was known suggested that this character is inherited as a simple one-factor recessive.

Although Snyder was the first to publish an account of parrot-beak as occurring in the United States of America, yet at that time a new recessive lethal had already been recorded by Annett (3) as occurring in milking Shorthorn cattle in New Zealand.

When Dr. Annett first informed me of the fact that he had noticed several fatal cases of defective lower jaw in the calves of the bull that he had purchased from me, I was loth to agree that inbreeding had brought to light an inherent defect in my bull. I was inclined to attribute the condition to malnutrition of the dam during pregnancy, possibly through mineral deficiency (4). I was confirmed in this belief because I could not remember a case of parrot-beak occurring in my herd, though for many years past I had been inbreeding very considerably. Shortly after this, however, a cow in my own herd gave birth to a calf with parrot-beak. This led me to make a careful search through my records, and, to my surprise, I found that a solitary case had occurred a few years previously. This case had not impressed itself on my memory; at the time I probably put it down to an accident in the development of the foetus, a somatic variation that was not hereditary. The sire of the two parrot-beak calves in my herd was Matangi Quality 4th's Boy. He was also the sire of Matangi Superfine 2nd, the bull referred to in Dr. Annett's paper.

I had sold Matangi Quality 4th's Boy's twin brother, Matangi Quality 4th's Lad, to Mr. G. D. Hall, Kiokio, Waikato, and on inquiry learned that descendants of this bull also had left parrot-beak calves. Figure 1 shows at

\* This paper was presented at the sixth annual conference of the New Zealand Society of Animal Production.

a glance the manner in which parrot-beak was transmitted in Mr. Hall's herd. Roman figures to the left indicate generations, Arabic figures locate individuals. Full particulars of these, herd-book numbers, breeders, &c., appear in the Appendix. Matangi Quality 4th's Lad (line 1, No. 4) was mated with cows of Coates' Herd Book, Australian Illawarra, and New Zealand non-pedigree origin. In brother-sister matings of the offspring, Pinedale Lad (line 2, No. 5), gave two parrot-beak calves, and Pinedale Quality Lad (line 2, No. 3) gave one. Pinedale Quality Lad also gave two parrot-beak calves (line 4, Nos. 2 and 3) from matings with Pinedale Lad's daughters. Matangi Quality 4th's Lad himself left a parrot-beak calf (line 4, No. 1) when mated with one of his granddaughters. Mr. Hall notified me of the birth of the latter calf; I was able to see it alive, and, after it was destroyed, photograph the head and lower jaw, with molars impacted and jaw-bone fractured (Figs. 2, 3, and 4).

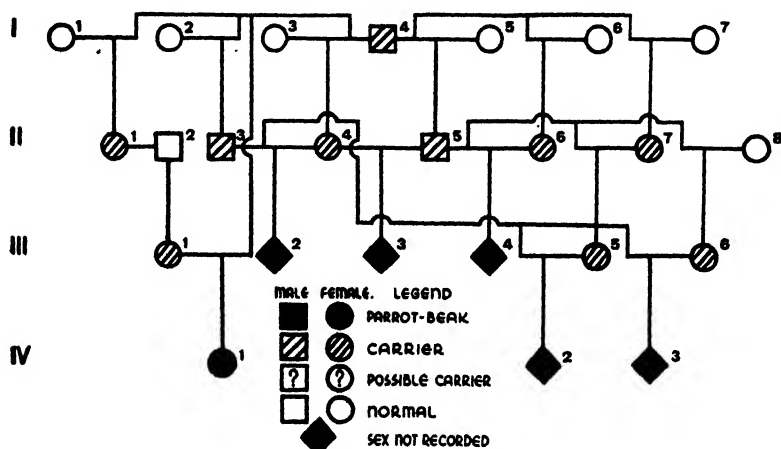


FIG. 1.—Family history of calves with parrot-beak. Bred by Mr. G. D. Hall, Kiokio, Waikato. (See Appendix for full details.)

Dr. Annett described the abnormality as follows: "A deformity of the lower jaw, which was only half the normal length. The teeth were normal and the tongue, having no support, hung straight down outside the mouth . . . such a calf cannot suck its dam, and, even if it survived, could not subsequently graze." When Dr. Annett described the teeth as normal, he evidently referred to the incisors, which were plainly visible; he had not thought of inspecting the molars (Fig. 4).

So Lerner(5), who had classified the parrot-beak of Heizer and Hervey as "A14, impacted molars," classified Annett's lethal as "A19, Agnathia." But on comparing the photographs of Hall's parrot-beak calf with those of Heizer and Hervey's it seems evident that the same lethal factor is concerned. In both cases the molars are impacted and the left side of the jaw-bone is fractured. Also, in both cases, the lethal appears to be inherited as a simple recessive.

In a private letter dated 28th September, 1938, giving particulars of the calves born in his herd during the five seasons ending 1935-36, Dr. Annett says: "Sixty-one cows not related to Matangi Superfine 2nd have given 155 calves by him. None of these calves have the undershot jaw. Thirty-one



FIG. 2.—Head of parrot-beaked calf showing the short lower jaw, which is approximately only half normal length.



FIG. 3.—Head of parrot-beaked calf showing shortened lower jaw and how the tongue, lacking support, hangs down outside the mouth.

of Superfine 2nd's own daughters have been put to Superfine 2nd and have given 56 calves. Of these 56 calves, 6 were noted as having undershot jaws." This mating should have given the 7:1 ratio—i.e., 49 normal to 7 parrot-beak; actually, 50 normal to 6 parrot-beak were born.

Then my brother, Thos. L. Ranstead, Matangi, told me that a parrot-beak calf had appeared in his herd some years previously. His cow, Matangi Ruth 2nd, held the New Zealand record for a senior three-year-old under C.O.R. test. He put her to my bull Matangi Quality 4th's Boy, whose dam held the world's record for a senior four-year-old under C.O.R. test. A

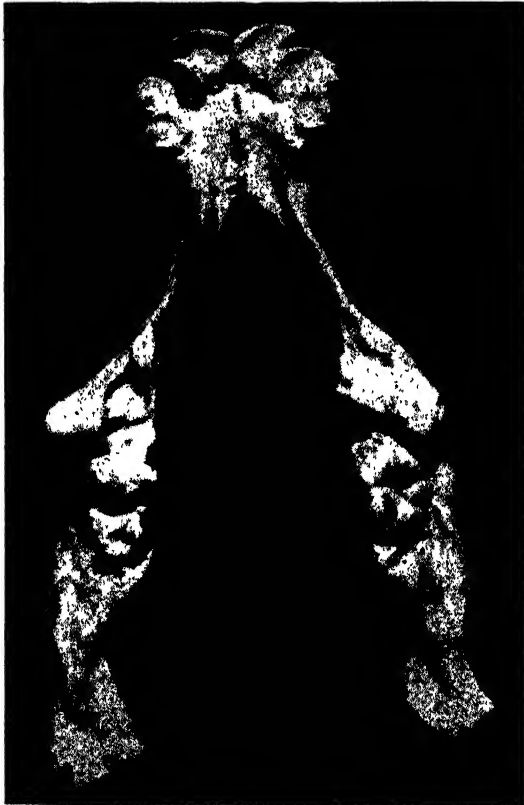


FIG. 4.—Lower jaw-bone of parrot-beaked calf showing the impacted molars and the fracture on the left side of the jaw-bone.

bull calf from this mating sold for 205 guineas, so it is easily understood how the mating came to be repeated four times. This resulted in five normal calves—3 male, 2 female. Two of these were mated, resulting in a parrot-beak female. This mating was repeated twice, resulting in two normal males.

In 1934 Miss Wotherspoon, Horsham Downs, Waikato, purchased a bull calf from me. This bull, when a little over a year old, was mated with six yearling heifers and six two-year-olds. When these commenced to calve in 1936 Miss Wotherspoon complained that all the calves were deformed.

The deformity in the calves was parrot-beak, so I at once knew that the bull that I had sold her was a carrier. I told Miss Wotherspoon that not all of the calves would be deformed. The chances were that the bull would hand on the factor to one-half of his offspring, and that these would not show the defect unless they also received the same recessive factor for parrot-beak from their dams, in which case her own cattle would be just as much to blame as my bull.

In order to obtain exact information I paid Miss Wotherspoon a visit, and the information I then gathered appears on the charts A and B in Fig. 5. I found that out of the twelve calves (B, line 3, Nos. 1 to 12) by my bull (B, line 2, No. 1) only three were deformed. The dams of the twelve calves (B, line 2, Nos. 2 to 6, born 1933, and Nos. 7 to 13, born 1934) were bred by Miss Wotherspoon, and were by a bull (B, line 1, No. 1) purchased from Mr. Hall. This bull and the bull that I sold to Miss Wotherspoon (B, line 2, No. 1) were related, their sires being the twin bulls Matangi Quality 4th's Lad and Matangi Quality 4th's Boy, which had proved themselves carriers.

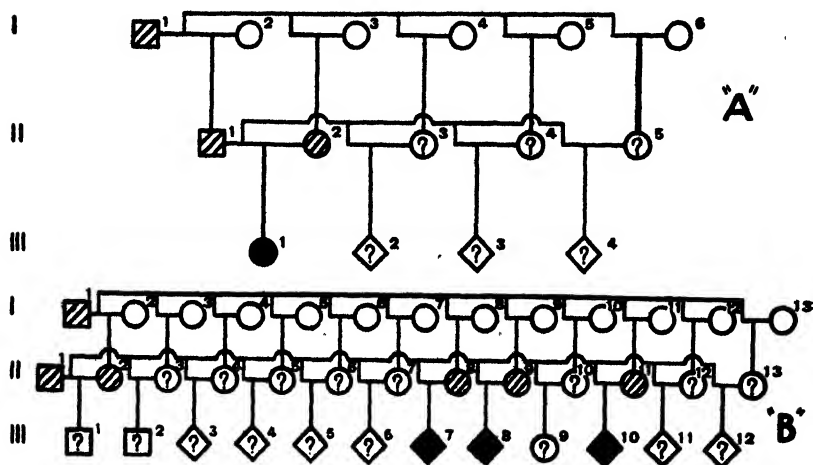


FIG. 5A and 5B.—Family history of calves with parrot-beak. Bred by Miss J. Wotherspoon, Horsham Downs, Waikato, New Zealand. (See Appendix for full details.)

in Mr. Halls' and my own herd. The bull purchased from Mr. Hall was evidently a carrier of the recessive factor, and so might be expected to pass it on to six of his twelve daughters. The bull purchased from me must also have been a carrier, and when mated with the twelve daughters, a 7 : 1 ratio could be expected—i.e., one parrot-beak in every eight calves born. Actually, double the expected number were born—one parrot-beak in every four births.

I also learned that a case of parrot-beak had occurred in Miss Wotherspoon's herd in 1935. Four of the above mentioned two-year-olds (B, line 2, Nos. 2 to 5) were mated the year before, when they were yearlings (A, line 2, Nos. 2 to 5), to a yearling bull (A, line 2, No. 1) bred on the farm by the same bull as themselves, Hall's bull (A, line 1, No. 1; also B, line 1, No. 1). This yearling bull that Miss Wotherspoon bred (A, line 2, No. 1, had evidently received the recessive factor from his sire, Hall's bull, and so had some of his half-sisters, for one of the four calves born from this mating had parrot-beak (A, line 3, No. 1). The following season the dam of this parrot-beak calf had a normal calf (B, line 3, No. 1) by my carrier bull.

Pedigrees are the basis of the study of heredity, but their value depends absolutely on the care with which the records are compiled. As many family histories as possible of the character to be studied, should be collected. They must then be carefully examined to see if a reasonable explanation can be given of the method of inheritance of the character concerned. I have collected four family histories of parrot-beak in pedigree milking Shorthorns. In Fig. 6, referring to the cattle of Ranstead Bros. (John M. Ranstead and Thos. L. Ranstead), the transmission of parrot-beak can be explained most readily by assuming that it is due to a simple autosomal recessive factor, introduced by the Coates' Herd Book bull Hippo (line 1, No. 2). As mutations are extremely rare, occurring around the magnitude of one mutation of each gene in 100,000 or 1,000,000 generations(6), it seems likely that Hippo, whose immediate ancestors were imported from England, inherited the factor from the same source as Heizer and Hervey's cases. These were also milking Shorthorns of English origin, and if records of their pedigrees were available, a comparison with Hippo's breeding would enable a check up

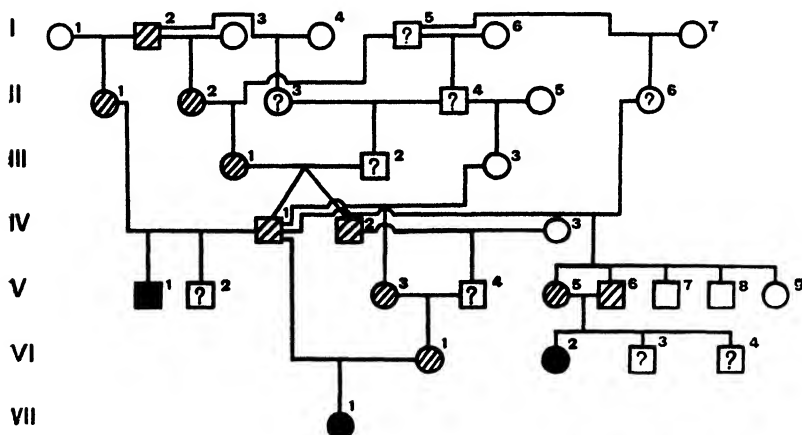


FIG. 6.—Family history of calves with parrot-beak. Bred by Ranstead Bros., Matangi, Waikato, New Zealand. (See Appendix for full details.)

to be made. When an explanation which seems reasonable has been assumed, it may be checked by making test matings. As the animals in Fig. 6 are all dead, it is not possible to make any test matings with them; but, as the most important of them were used extensively for breeding purposes while alive and very accurate records were kept, it is possible by looking through the records to select suitable matings for checking purposes.

Running back through the chart (Fig. 6), from the parrot-beak calf No. 1 on line 7, there is no doubt that Quality 4th's Boy is a carrier; but there is a doubt about Pinedale Boss, No. 4 on line 5. On checking his records it is found that he was mated with twenty daughters of Quality 4th's Boy, who was a carrier. From such matings, if Boss were a carrier, one calf in eight, the 7 : 1 ratio, should have exhibited parrot-beak. Actually, from these matings, Boss left 35 normal calves. Mated with his own daughter, Boss left a normal calf, and mated with other relations in the herd, from seven cows he left 10 normal calves. So I have assumed that Boss did not carry the factor. There is also a doubt about Jewel's Prince, No. 2 on line 3, the sire of Quality 4th's Boy. It is recorded that Quality

4th's Boy, a carrier, was mated with nine daughters of Jewel's Prince, and these matings produced 54 calves. If Jewel's Prince had been a carrier, one in eight of these calves should have exhibited parrot-beak. All were normal. So I have assumed that Jewel's Prince did not carry the factor. Going back still further, Matangi Prince was checked up (No. 4, line 2). Quality 4th's Boy, a carrier, mated with six daughters of Prince, left 38 normal calves instead of parrot-beaks in the 7:1 ratio which would have been expected if Prince had been a carrier. So I have assumed that Prince did not carry the factor. Going back to Dominion Esau of Ruakura (No. 5, on line 1) it is recorded that seven of his daughters were mated with Quality 4th's Boy, the known carrier, and 15 normal calves were born instead of parrot-beak calves in the 7:1 ratio. So I have assumed that Esau was not a carrier. As Nos. 1 and 2 are of Coates' Herd Book origin and No. 3 is of unknown parentage, and having reached line 1 and eliminated all sources of parrot-beak but Nos. 1, 2, and 3, the odds seem to be 2 to 1 on Coates' Herd Book. As it is unlikely that both Nos. 1 and 2 should introduce such a rare factor, and as No. 2 must have introduced it if No. 3 is cut out, I have assumed that No. 2 (Hippo) is the guilty animal.

In cattle-breeding the smallness of the numbers involved affects the statistical examination of the results. Expected ratios are rarely observed. For instance, Matangi Quality 4th's Boy, who was a carrier, when mated with twelve of his own daughters, left 20 normal calves. Mated with ten half-sisters, he left 58 normal calves. If no further matings with near relations had taken place, parrot-beak would not have appeared in my herd. But when mated with five granddaughters he left 8 calves—7 normal and 1 with parrot-beak. Again, when mated with two daughters of his great-grandfather, he left 3 calves—2 normal and 1 with parrot-beak.

Though the above results seem very erratic, when they are considered together with those of Dr. Annett, Miss Wotherspoon, and Thos. L. Ranstead (Mr. Hall's results are not suitable for consideration because the number of normal calves was not given), a different picture is presented. I have arranged below, in tabular form, all the matings, in the four herds under consideration, of known carrier bulls with known and possible carrier cows, and the results give nearly the expected ratios. In the matings shown, 67 cows gave 106 calves. The theoretical expectancy of 91.48 normal to 14.52 parrot-beak calves is closely approximated by the actual result of 93 normal and 13 parrot-beak.

TABLE I.—INHERITANCE OF PARROT-BEAK IN MATINGS OF KNOWN CARRIER BULLS WITH KNOWN AND POSSIBLE CARRIER COWS

Owners.	Matings.				Calves.					
	Bulls (H.B. No.).	Cows.		No.	No.	Expected.			Actual.	
		Relation to Bull.	No.			Ratio.	Normal.	Parrot-beak.	Normal.	Parrot-beak.
Dr. Annett ..	5013	Daughters ..	31	56	7:1	49	7	50	6	
Thos. L. Ranstead ..	7022	Full sister (carrier)	1	3	3:1	1.75	1.25	2	1	
John M. Ranstead ..	4245	Daughter of great-grandfather (carrier)	1	2	3:1	1.5	0.5	1	1	
		Daughter of great-grandfather	1	1	7:1	0.875	0.125	1	0	
		Daughters ..	12	20	7:1	17.5	2.5	20	0	
		Granddaughters ..	5	8	6:1	6.857	1.143	7	1	
Miss J. Wotherspoon	8147	Half-sisters ..	4	4	7:1	3.5	0.5	3	1	
	7941	Daughters of 6659	12	12	7:1	10.5	1.5	9	3	
			67	106	..	91.48	14.52	93	13	

Quality 4th's Boy was used for eleven seasons, leaving 195 normal and 2 parrot-beak calves. During that period my herd averaged 37 head of cows in milk, all of which had been bred on the farm and were related to Quality 4th's Boy. After him, his son, grandson, great-grandson, great-great-grandson, and great-great-great-grandson have been used in my herd, leaving no parrot-beak calves. I have only dealt in small numbers, and luck has been on my side.

My experience has a bearing on artificial insemination, for which work a bull is selected after a relatively small number of matings. Sires for this work are proven on a basis of butterfat production only, usually in the herd of one owner, and it does not seem possible to prove a bull for lethals in one herd at such an age that he could be used for artificial insemination work. If a bull selected for artificial insemination work carried a lethal factor, and if he were followed by a carrier son, following the advice that, "The next best bull to a proven sire is the son of a proven sire" (7), the total of the large number of matings would probably give the expected ratio of normal to lethal. But the various groups of these matings would not do so. In some herds the lethal might not come to expression, in others, what appeared to be an epidemic, might occur.

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- (6) LUSH, J. L. (1937): "Animal Breeding Plans," p. 52.
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#### APPENDIX

Numbers refer to the New Zealand Milking Shorthorn Herd-book unless otherwise stated.

N.Z.S. = New Zealand Shorthorn Herd Book, which only admits cattle eligible for Coates' Herd Book.

Darbalara = from the Darbalara Herd of Australian Illawarra Shorthorns, not eligible for N.Z.S. or Coates' Herd Book.

S.N. = Station number, owner's private brand.

#### FIGURE 1

Line.	No.	
I	1	<i>Female: Glenbank Princess 2nd, 17863</i> ; colour red; born Aug., 1924; bred by Mr. Jno. Nicol, Ardmore, Auckland; by Onewhero Rufus, 2892, whose sire and dam were bred by Ranstead Bros.
	2	<i>Female: Glenbank Rose 2nd, 16268</i> ; colour red; born July, 1923; bred by Mr. Jno. Nicol, Ardmore, Auckland; by Onewhero Rufus, 2892.
	3	<i>Female: Pinedale Bel!, 15538</i> ; colour red and white; born Aug., 1923; bred by Mr. G. D. Hall; by Field Marshal of Cornwall Park, 709; bred by Mr. J. C. Wright, Matapu, Taranaki. Field Marshal of Cornwall Park was by Acorn of Oakdale, 46 (imported Australian Illawarra). Field Marshal was sold to Mr. W. Gadsby, Te Kuiti, Auckland, at the age of 14 months for 205 guineas.
	4	<i>Male: Matangi Quality 4th's Lad, 4246</i> ; colour roan; born June, 1925; bred by John M. Ranstead.
	5	<i>Female: Glenbank Plum, 12345</i> ; colour red; born Aug., 1919; bred by Mr. Jno. Nicol, Ardmore, Auckland; by Hukerewai Count 3rd, 4033 N.Z.S.; bred by Mr. Alex. D. Bell, Clevedon, Auckland.



## APPENDIX—continued

## FIGURE 1—continued

Line.	No.	
	6	<i>Female : Glenbank Rose 2nd, 16268 ; colour red ; born July, 1923 ; bred by Mr. Jno. Nicol, Ardmore, Auckland, by Onewhere Rufus, 2892.</i>
	7	<i>Female : Waitangi Rosebloom 2nd, 17666 ; colour red and white ; born Aug., 1925 ; bred by Mr. A. J. McGovern, Kiokio, Auckland ; by Waitangi Marshal, 2120, whose sire and dam were imported from Darbalara, Australia.</i>
II	1	<i>Female : Pinedale Dot, 19284 ; colour light roan ; born July, 1927.</i>
	2	<i>Male : Riverdale Goldmine 44th, 5125 ; colour red ; born Sept., 1927 ; bred by Mr. T. W. Wardlaw, Waimana, Bay of Plenty ; by Pine Farm Goldmine, 2885, out of Riverdale Florrie 2nd, 8570. Pine Farm Goldmine was by Marlborough of Darbalara, 110 (imp.), out of Lilly, 96, a non-pedigree cow that held the New Zealand record for the breed in the early days of the C.O.R. test.</i>
	3	<i>Male : Pinedale Quality Lad, 7591 ; colour roan ; born Aug., 1933.</i>
	4	<i>Female ; Pinedale Rona, 19300 ; colour light roan ; born July, 1927.</i>
	5	<i>Male : Pinedale Lad, 4734 ; colour dark red ; born July, 1927.</i>
	6	<i>Female : Pinedale Rosette, 21677 ; colour red, little white ; born July, 1929.</i>
	7	<i>Female : Pinedale Rosebloom, 23962 ; colour roan ; born Aug., 1930.</i>
	8	<i>Female : Pinedale Rabbit, A-249 ; colour red ; born 1917 ; pedigree unknown.</i>
III	1	<i>Female : Pinedale Doris, 27926 ; colour roan ; born June, 1935.</i>
	2	<i>(Parrot-beak) ; sex, colour, and date of birth not recorded.</i>
	3	<i>(Parrot-beak) ; sex, colour, and date of birth not recorded.</i>
	4	<i>(Parrot-beak) ; sex, colour, and date of birth not recorded.</i>
	5	<i>Female : Pinedale Rosebloom 2nd, 27957 ; colour red, little white ; born Aug., 1934.</i>
	6	<i>Female : Pinedale Babs, A-1410 ; colour red, born Aug., 1931.</i>
IV	1	<i>Female (parrot-beak) ; colour roan ; born Nov., 1938. The head and lower jaw, with molars impacted and jaw-bone fractured, were photographed.</i>
	2	<i>(Parrot-beak) ; sex, colour, and date of birth not recorded.</i>
	3	<i>(Parrot-beak) ; sex, colour, and date of birth not recorded.</i>

## FIGURE 5A

I	1	<i>Male : Pinedale Daisy Boy, 6659 ; colour roan ; born July, 1930 ; bred by Mr. G. D. Hall ; by Matangi Quality 4th's Lad, 4246, bred by John M. Ranstead.</i>
	2	<i>Female : Bon Accord Doris, A-2638 ; colour roan ; born 1931 ; bred by Miss Wotherspoon ; by Muriwai Pride, 4635.</i>
	3	<i>Female : Bon Accord Brownie, A-2636 ; colour red ; born 1931 ; bred by Miss Wotherspoon ; by Muriwai Pride, 4635.</i>
	4	<i>Female : No particulars recorded.</i>
	5	<i>Female : No particulars recorded.</i>
	6	<i>Female : No particulars recorded.</i>
II	1	<i>Male : Bon Accord Captain, 8147 ; colour red ; born Sept., 1933.</i>
	2	<i>Female : Bon Accord Peggy, A-3314 ; colour roan ; born June, 1933.</i>
	3	<i>Female : Name and colour not recorded ; born 1933.</i>
	4	<i>Female : Name and colour not recorded ; born 1933.</i>
	5	<i>Female : Name and colour not recorded ; born 1933.</i>
III	1	<i>Female (parrot-beak) ; colour not recorded ; born 1935.</i>
	2	<i>? Normal calf ; sex and colour not recorded ; born 1935.</i>
	3	<i>? Normal calf ; sex and colour not recorded ; born 1935.</i>
	4	<i>? Normal calf ; sex and colour not recorded ; born 1935.</i>

## FIGURE 5B

I	1	<i>Male : Pinedale Daisy Boy, 6659 ; colour roan ; born July, 1930 ; bred by Mr. G. D. Hall ; by Matangi Quality 4th's Lad, 4246, bred by John M. Ranstead.</i>
	2	<i>Female : Bon Accord Brownie, A-2636 ; colour red, born 1931 ; bred by Miss Wotherspoon ; by Muriwai Pride, 4635.</i>
	3	<i>Female : Bon Accord Pet, A-2639 ; colour roan ; born 1929 ; bred by Miss Wotherspoon ; by Muriwai Pride, 4635.</i>

## APPENDIX—continued

Line.	No.	FIGURE 5B—continued
	4	Female: No particulars recorded.
	5	Female: No particulars recorded.
	6	Female: No particulars recorded.
	7	Female: No particulars recorded.
	8	Female: <i>Bon Accord Pet, A-2639</i> ; colour roan; born 1929; bred by Miss Wotherspoon; by Muriwai Pride, 4635.
	9	Female: <i>Bon Accord Rosemary, A-1843</i> ; colour roan; born 1930; bred by Miss Wotherspoon; by Muriwai Pride, 4635.
	10	Female: <i>Bon Accord Rosie, A-1257</i> ; colour red; born 1928; bred by Miss Wotherspoon; by Hillgrove Duke 24th, 4281.
	11	Female: No particulars recorded.
	12	Female: No particulars recorded.
	13	Female: No particulars recorded.
II	1	Male: <i>Matangi Superfine 54th, 7941</i> ; colour red; born Aug., 1934; bred by John M. Ranstead; by Matangi Quality 4th's Boy, 4245.
	2	Female: <i>Bon Accord Peggy, A-3314</i> ; colour roan; born June, 1933.
	3	Female: <i>Bon Accord Gem, 29130</i> ; colour roan; born Sept., 1933.
	4	Female: Name and colour not recorded; born 1933.
	5	Female: Name and colour not recorded; born 1933.
	6	Female: Name and colour not recorded; born 1933.
	7	Female: Name and colour not recorded; born 1933.
	8	Female: <i>Bon Accord Mermaid, 29132</i> ; colour dark red; born July, 1934.
	9	Female: <i>Bon Accord Rosamond 2nd, 29133</i> ; colour roan; born Aug., 1934.
	10	Female: <i>Bon Accord Rosebud, 29134</i> ; colour roan; born June, 1934.
	11	Female: <i>Bon Accord Pansy</i> ; not entered in herd book; colour not recorded; born 1934.
	12	Female: Name and colour not recorded; born 1934.
	13	Female: Name and colour not recorded; born 1934.
III	1	Male: <i>Bon Accord Super, 10280</i> ; colour red; born 1936.
	2	Male: <i>Bon Accord General, 10277</i> ; colour red and white; born Oct., 1936.
	3	? Normal calf; sex and colour not recorded; born 1936.
	4	? Normal calf; sex and colour not recorded; born 1936.
	5	? Normal calf; sex and colour not recorded; born 1936.
	6	? Normal calf; sex and colour not recorded; born 1936.
	7	(Parrot-beak); sex and colour not recorded; born 1936.
	8	(Parrot-beak); sex and colour not recorded; born 1936.
	9	Female: <i>Bon Accord Rosebud 2nd, 31519</i> ; colour red; born Sept., 1936.
	10	(Parrot-beak); sex and colour not recorded; born 1936.
	11	? Normal calf; sex and colour not recorded; born 1936.
	12	? Normal calf; sex and colour not recorded; born 1936.

## FIGURE 6

I	1	Female: <i>Matangi Lilly, 2033</i> ; colour roan; born Aug., 1910; bred by Ranstead Bros.; by Coates' Herd Book bull bred by Mr. A. R. Winstone, Mount Roskill, Auckland.
	2	Male: <i>Hippo, 2944 N.Z.S.</i> ; colour roan; born Sept., 1913; bred by Hon. John Fisher, Pukerimu, Waikato; by Commissioner, 2390 N.Z.S., bred by Mr. A. Chamberlain, Ellesmere, Canterbury, by Ambush (imp.), 87808 Coates' Herd Book.
	2	Female: <i>Matangi Quartus, 2615</i> ; colour roan; born 1907; breeder unknown, foundation cow.
	4	Female: <i>S.N. 139</i> ; colour red; born 1911; bred by Ranstead Bros.; by a son of Rachel's Lad, 2346 N.Z.S., bred by Canterbury Agricultural College.
	5	Male: <i>Dominion Esau of Ruakura, 559</i> ; colour red; born 20th Feb., 1916; bred by Ruakura Government Farm; by Dominion Abram 2nd of Ruakura (imp. from Darbalara).
	6	Female: <i>Maniaroa Princess, 6939</i> ; colour roan; born 1912; bred by Mr. A. C. Hoyer, Tauwhare, Waikato; by Red Light, 2176 N.Z.S., bred by Ruakura Government Farm, by Red Lord 17th (imp.), 79713 Coates' Herd Book, bred by Mr. J. T. Hobbs, Maiseyhampton, England; Red Light out of Delight (imp.), 3335 N.Z.S., bred by Mr. G. Gerrard, Offertin Farm, Hindlip, Worcester, England. Maniaroa Princess: C.O.R. 15,951 lb. milk, 4.4 test, 700.76 lb. fat, first Shorthorn to exceed 700 lb. fat in New Zealand.

## APPENDIX—continued

## FIGURE 6—continued

Line.	No.	
	7	<i>Female: Matangi Rachael, 2035</i> ; colour red; born 1909; bred by Newcombe Bros., Karapiro, Cambridge, Waikato; non-pedigree, foundation cow.
II	1	<i>Female: Matangi Lilly 3rd, 12536</i> ; colour white; born 9th Sept., 1916; bred by Ranstead Bros.
	2	<i>Female: Matangi Quality, 2033</i> ; colour roan; born Aug., 1915; bred by Ranstead Bros.
	3	<i>Female: Matangi Jewel, 2020</i> ; colour red; born Aug., 1915; bred by Ranstead Bros.; C.O.R. as four-year-old, 12,524 lb. milk, 4.0 test, 509 lb. fat.
	4	<i>Male: Matangi Prince, 2925</i> ; colour roan; born Feb., 1921; bred by Ranstead Bros.
	5	<i>Female: Matangi Elizabeth, 2009</i> ; colour roan; born Aug., 1913; bred by Ranstead Bros.; by Ruakura S.N. 112, bred by Ruakura Government Farm, by Red Light, 2176 N.Z.S.
	6	<i>Female: Matangi Ruth 2nd, 10642</i> ; colour red; born Oct., 1918; bred by Ranstead Bros.; C.O.R. 14,032 lb. milk, 5.3 test, 747 lb. fat, New Zealand record for senior three-year-old.
III	1	<i>Female: Matangi Quality 4th, 10640</i> ; colour roan; born Aug., 1919; bred by Ranstead Bros.; C.O.R. 22,010 lb. milk, 4.4 test, 978 lb. fat. World's record for senior four-year-old, New Zealand record for the breed, all ages.
	2	<i>Male: Matangi Jewel's Prince, 3570</i> ; colour red; born Sept., 1923; bred by J. M. Ranstead.
	3	<i>Female: Matangi Princess Elizabeth, 18009</i> ; colour roan; born Sept., 1923; bred by John M. Ranstead.
IV	1	<i>Male: Matangi Quality 4th's Boy, 4245 (twin)</i> ; colour red; born June, 1925; bred by John M. Ranstead.
	2	<i>Male: Matangi Quality 4th's Lad, 4246 (twin)</i> ; colour roan; born June, 1925; bred by John M. Ranstead; sold to Mr. G. D. Hall, Kiokio, Waikato.
	3	<i>Female: Waitangi Blossom, 17646</i> ; colour roan; born Aug., 1925; bred by Mr. A. J. McGovern, Kiokio; line bred to Alexander of Darbalara (imp.), 151; sold to Mr. G. D. Hall.
V	1	<i>Male (parrot-beak)</i> ; colour roan; born Sept., 1928; bred by John M. Ranstead.
	2	<i>Male: Matangi Superfine 20th, 6064</i> ; colour roan; born Sept., 1929; bred by John M. Ranstead; sold to Mr. F. Hull, Waiuku, Auckland.
	3	<i>Female: Matangi Princess Elizabeth 3rd</i> ; colour red; born Oct., 1928; bred by John M. Ranstead.
	4	<i>Male: Pinedale Boss, 5584</i> ; colour white; born Dec., 1928; bred by Mr. G. D. Hall, Kiokio.
	5	<i>Female: Matangi Ruth 12th, 20198</i> ; colour red; born Nov., 1927; bred by T. L. Ranstead.
	6	<i>Male: Matangi Bullion, 7022</i> ; colour red; born Nov., 1930; bred by T. L. Ranstead.
	7	<i>Male: Matangi Ruth 2nd's Boy, 6044</i> ; colour red; born Oct., 1928; bred by T. L. Ranstead.
	8	<i>Male: Matangi Ace, 6006</i> ; colour red; born Oct., 1929; bred by T. L. Ranstead; sold to Mr. T. Wardlaw, Waimana, Bay of Plenty.
	9	<i>Female: Matangi Ruth 17th, 26737</i> ; colour red; born July, 1932; bred by T. L. Ranstead.
VI	1	<i>Female: Matangi Princess Elizabeth 6th, 24733</i> ; colour roan; born Sept., 1931; bred by John M. Ranstead.
	2	<i>Female (parrot-beak)</i> ; colour red, born Nov., 1930; bred by T. L. Ranstead.
	3	<i>Male: Matangi Tom, 7942</i> ; colour red; born Aug., 1934; bred by T. L. Ranstead.
	4	<i>Male: (Not entered in herd book)</i> ; colour red; born July, 1936; bred by T. L. Ranstead.
VII	1	<i>Female (parrot-beak)</i> ; colour red; born Aug., 1935; bred by John M. Ranstead.

EFFECT OF CERESAN ON THE GERMINATION OF  
STORED LINEN-FLAX SEED

By M. A. BLACK, Agronomy Division, Plant Research Bureau, Lincoln

[Received for Publication, 20th August, 1946]

*Summary*

Ceresan treatment, wet or dry, has not reduced the germination of linen-flax seed stored at moderate temperature and low relative humidity for three and a half years.

In view of the widely held belief that seed treated with organic mercurials cannot be stored for more than a few months without serious reduction in germinating ability, it is thought that the following note may be of general interest.

Seed has been stored in a steel filing cabinet in an occupied office at the Agronomy Division, Lincoln, for a number of years, and a sample of linen-flax seed has germinated 100 per cent. in three days after six and a half years in this cabinet. The temperature and relative humidity are not accurately known, but it is thought that the temperature would rarely, if ever, fall below 55° F. or rise above 75° F., and the average relative humidity is probably in the neighbourhood of 50 per cent. At 60° F. and 60 per cent. relative humidity the equilibrium moisture content (M.C.) of flax-seed is about 8.6 per cent. Larmour *et al.*(1) state that in America 10.5 per cent. M.C. is considered the maximum for safe storage of flax-seed.

The moisture contents given below are approximate, as no vacuum oven was available, and the figures are actually loss of weight on drying in an ordinary oven for twenty-four hours at 105° C.

The germination tests were made on wet blotting-paper in covered petri dishes at 70° F. to 72° F. on single samples of 100 seeds.

Line A was Concurrent seed from the 1940-41 harvest with a germination in June, 1941, of 96 per cent. in two days and 97 per cent. in seven days. In January, 1943, part of this line was heavily dusted with Ceresan New, the surplus dust being lightly screened off, leaving a dressing of 4.2 oz. per bushel, measured by the difference in weight before and after dusting.

The moisture content of the control, and the germination of control and dusted seed before and after three and a half years' storage under the conditions described above, is given in Table I.

TABLE I

	January, 1943.			August, 1946.		
	M.C. (per Cent.).	Germination (per Cent.).		M.C. (per Cent.).	Germination (per Cent.).	
		Two Days.	Seven Days.		Three Days.	Seven Days.
A. Control ..	8.6	95	96	8.1	92	93
Ceresan dusted ..	..	95	96	..	93	95

Line B was Concurrent seed from the 1942-43 harvest, and part was sprayed with water-soluble Ceresan U. 564 by the "short wet" method and stored as above.

The moisture content and germination figures are given in Table II.

TABLE II

	July, 1943.			August, 1946.		
	M.C. (per Cent.).	Germination (per Cent.).		M.C. (per Cent.).	Germination (per Cent.).	
		Two Days.	Seven Days.		Three Days.	Seven Days.
B. Control ..	9.6	99	99	8.1	99	99
Ceresan sprayed ..	..	96	99	..	100	..

## REFERENCE

- (1) LARMOUR, R. K.; SALLANS, H. R.; and CRAIG, B. M. (1944): "Respiration of Whole and Dehulled Sunflower-seed and of Flax-seed." *Can. J. Res.*, 22, 9-18.

## A NOTE ON PROGENY TESTING

PROPORTION OF RAMS WHICH CAN BE PROGENY TESTED IN  
NEW ZEALAND ROMNEY MARSH STUD FLOCKS

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Agricultural College, University of New Zealand, Palmerston North

[Received for publication, 24th May, 1946]

## Summary

A theoretical table is given showing the number of ewes required to leave from 7 to 30 progeny per sire, for progeny testing various proportions of the yearly crop of rams.

In view of the importance of progeny testing it might be well to consider briefly the question of how many rams can be progeny tested under commercial stud conditions obtaining in New Zealand (Manawatu District).

A hypothetical stud containing 1,000 breeding-ewes will be considered. Apart from limiting factors of general management such as the number of paddocks available, labour, &c., the number of rams which can be progeny tested would, broadly speaking, depend on—

- Lambing percentage and sex ratio:
- Mortality and culling of progeny (mostly on "fancy points" and malformations) before first shearing:
- The number of progeny needed for a sire's appraisal:
- The subdivision of the stud-flock.

As it is hoped to show elsewhere (Goot, 1946c) that the figures taken into account while preparing Table I are based on the following approximate averages available from stud A (Goot, 1946A) for lambing percentages, culling, and mortality:—

- (a) Lambing percentage of 100–110 per cent. at docking and approximately 90 per cent. at the age of twelve to fourteen months (an approximately equal sex ratio)  
 (b) Culling, mortality (including missing sheep), &c., 20 per cent. for ♂ ♂ and 13 per cent. for ♀ ♀

For example, the expected number of rams docked .. 500  
 20 per cent. deduction due to culling, mortality, &c. .. 100

Total rams available .. .. 400

TABLE I.—NUMBER OF EWES REQUIRED TO LEAVE VARIOUS NUMBERS OF PROGENY WHEN DIFFERENT PROPORTIONS OF YEARLY CROP OF RAMS ARE TO BE PROGENY TESTED. A HYPOTHETICAL STUD CONTAINING 1,000 BREEDING-EWES

Number of Ewes required to leave.	Rams to be progeny tested.*								Average Number of Ewes per Ram.
	20	15	10	8	6	4	2	1	
Percentage of rams ..	20	15	10	8	6	4	2	1	
Number of rams ..	80	60	40	32	25	16	8	4	
7 progeny* ..	624	468	312	250	195	125	62	31	7.8
10 progeny ..	888	666	444	355	278	178	89	44	11.1
12 progeny ..	1,064	798	532	426	333	213	106	53	13.3
15 progeny ..	1,320	990	660	528	413	264	132	66	16.5
18 progeny ..	1,600	1,200	800	640	500	320	160	80	20.0
20 progeny ..	1,776	1,332	888	710	555	355	178	89	22.2
25 progeny ..	2,224	1,668	1,112	890	695	445	222	111	27.8
30 progeny ..	2,664	1,998	1,332	1,066	833	533	266	133	33.3

\* Calculated on sheep which are expected to reach hogget age.

Similarly, the numbers of dams required to leave different numbers of offspring were calculated on the basis of 90 per cent. of the progeny attaining the hogget age—viz., for, say, 10 progeny 11 ewes are needed per ram, on the average. In this way all the main factors responsible for the proportion of rams which can be progeny tested have in Table I been reduced to two—namely, the proportion of rams to be progeny tested and the number of progeny required per sire.

The method of progeny testing requires a high proportion of two-tooth, and, if possible, ram hoggets to be tested and culled. McMahon (1940), for instance, calculated the following figures for the quickest and greatest improvement of progeny in regard to the fleece weight: “culling 80 per cent. rams and 50 per cent. ewes; progeny test 20 per cent. rams, keep 3 per cent.”

With regard to the number of progeny per sire, various workers (Vasin, 1934; McMahon, 1940; Ensminger *et al.*, 1943; and others) have postulated figures of progeny ranging from seven to well over one hundred. Workers of this Department regard fifteen to twenty offspring as desirable, especially as nearly half of the progeny will be of the opposite sex and the ram hoggets are usually “done” better than the ewe hoggets.

Confining the analysis of figures given in Table I to a simple arithmetical aspect, it is evident that the proportion of rams which can be progeny tested varies inversely with the number of offspring sired. Assuming that 3 per cent. of progeny-tested rams are to be kept for breeding as stud sires (McMahon, 1940) and each of them is mated to fifty ewes on the average (Goot, 1946B), it is evident that some six hundred ewes ( $50 \text{ ewes} \times 12 \text{ sires} = 600 \text{ ewes}$ ) are needed for stud breeding, leaving some 400 ewes for the purpose of progeny testing. Taking an average of fifteen progeny per ram it appears that no more than, say, 4-6 per cent. of the yearly crop of rams could be progeny tested.

A subdivision of the stud-flock into a "top stud" and "stud" might be advantageous. In this case the rams to be progeny tested would be selected from the "top stud" only. By doing this the integrity and continuity of a stud's characteristics could be better preserved. It is, however, outside the scope of the present note to discuss more fully the rather involved question of practical application of progeny testing of sheep so as to fit into the pattern of stud management, policy, and commercial requirements of ram-breeding in this country. In the writer's opinion, there is room for fuller inquiry into and a better understanding of even this simple aspect of progeny testing.

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SECOND SUPPLEMENT TO "A LIST OF PLANT  
DISEASES RECORDED IN NEW ZEALAND"

By R. M. BRIEN, Mycologist, Plant Diseases Division, Department of  
Scientific and Industrial Research

[Received for publication, 15th September, 1946]

IN the following list are recorded 54 diseases of fungous, bacterial, virus, and physiological origin from 56 host-plants. These include either diseases reported for the first time, or additional host records, since publication of the original "List of Plant Diseases Recorded in New Zealand" (Brien (1939) ), and the "First Supplement" (Brien (1942) ). No records of diseases on artificially infected host-plants have been included.

<b>Agropyron scabrum</b> (Lab.) Beauv. <i>Claviceps purpurea</i> (Fr.) Tul. .. ..	Neill, 1941.
<b>Ammophila arenaria</b> (L.) Link. <i>Claviceps purpurea</i> (Fr.) Tul. .. ..	Neill, 1941.
<b>Antirrhinum majus</b> L. <i>Peronospora antirrhini</i> Schroet. .. ..	New record.
<b>Atropa belladonna</b> L. <i>Verticillium dahliae</i> Kleb. .. ..	Additional host record.
<b>Beta vulgaris</b> L. Sugar-beet mosaic— <i>Beta virus</i> 2 of Smith, 1937 ..	Matthews, 1945.
<b>Brassica oleracea</b> L. <i>Cylindrosporium concentricum</i> Grev. .. ..	New record.
Black leaf-speck (physiological) .. ..	New record.
<b>Brassica oleracea</b> L. var. <b>botrytis</b> L. <i>Cylindrosporium concentricum</i> Grev. .. ..	Cunningham, 1944.
Whiptail (physiological) .. ..	Mitchell, 1945.
<b>Bromus inermis</b> Leyss. <i>Claviceps purpurea</i> (Fr.) Tul. .. ..	Neill, 1941.
<b>Calendula officinalis</b> L. <i>Entyloma calendulae</i> (Oud.) de Bary. .. ..	Cunningham, 1945c.
<i>Puccinia distincta</i> McAlp. .. ..	Cunningham, 1945a.
<b>Camellia japonica</b> L. <i>Pestalotia guepini</i> Desm. .. ..	New record.
<b>Carex dissita</b> Sol. <i>Farysia pseudocyperi</i> (de Toni) Zundel. ..	Cunningham, 1945b.
<b>Carex riparia</b> Curt. <i>Farysia olivacea</i> (DC.) Syd. .. ..	Cunningham, 1945b.
<b>Carex ternaria</b> Forst. f. <i>Farysia olivacea</i> (DC.) Syd. .. ..	Cunningham, 1945b.
<b>Carpha alpina</b> R. Br. <i>Cintractia waiouru</i> G. H. Cunn. .. ..	Cunningham, 1945c.



<b>Citrus</b> sp. (N.Z. grapefruit).			
<i>Phytophthora citrophthora</i> (Smith & Smith) Leon.			Additional host record.
<b>Clematis</b> <i>afoliata</i> Buch.			
<i>Aecidium otagenae</i> Linda.	..	..	Cunningham, 1945A.
<b>Coronopus</b> <i>didymus</i> (L.) Sm.			
<i>Peronospora parasitica</i> (Pers.) Tul.	..	..	Cunningham, 1944.
<b>Cucurbita</b> <i>pupo</i> L.			
<i>Erysiphe cichoracearum</i> DC.	..	..	New record.
<i>Mycosphaerella citrullina</i> (C.O.Sm.) Grossenb.	..	..	Additional host record.
<b>Cyphomandra</b> <i>betacea</i> Sendt.			
<i>Glomerella cingulata</i> (Stonemn.) Spauld. & V. Schr.			Additional host record.
<i>Oidium</i> sp.	..	..	New record.
<b>Danthonia</b> <i>pliosa</i> R. Br.			
<i>Ustilago comburens</i> Ludw.	..	..	Cunningham, 1945c.
<b>Daphne</b> <i>odora</i> Thunb.			
Daphne-mosaic (not classified)	..	..	Chamberlain & Matthews, 1941.
<b>Deyeuxia</b> <i>quadriseta</i> (Labill.) Benth.			
<i>Tilletia inolens</i> McAlp.	..	..	Cunningham, 1945c.
<b>Dipsacus</b> <i>fullonum</i> L.			
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	..	..	Additional host record.
<b>Eriobotrya</b> <i>japonica</i> Lindl.			
<i>Fabraea maculata</i> (Lev.) Atk.	..	..	Additional host record.
<b>Escallonia</b> <i>maerantha</i> Hook. & Arn.			
<i>Stereum purpureum</i> Pers.	..	..	Additional host record.
<b>Festuca</b> <i>arundinacea</i> Schreb.			
<i>Urocystis agropyri</i> (Preuss) Schroet.	..	..	Cunningham, 1945c.
<b>Ficus</b> <i>carica</i> L.			
Fig-mosaic— <i>Ficus virus</i> 1 of Smith, 1937	..	..	Li & Procter, 1944.
<b>Gahnia</b> <i>setifolia</i> (A. Rich.) Hook. f.			
<i>Farysia endotricha</i> (Berk.) Syd.	..	..	Cunningham, 1945B.
<b>Gahnia</b> <i>xanthocarpa</i> Hook. f.			
<i>Farysia endotricha</i> (Berk.) Syd.	..	..	Cunningham, 1945B.
<b>Gerbera</b> sp. cult.			
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary.	..	..	Additional host record.
<b>Glyceria</b> <i>fluitans</i> (L.) R. Br.			
<i>Claviceps purpurea</i> (Fr.) Tul.	..	..	Neill, 1941.
<b>Hebe</b> <i>salicifolia</i> (Forst. f.) Penn.			
<i>Aecidium disciforme</i> McAlp.	..	..	Cunningham, 1945A.
<b>Hoseyamus</b> <i>niger</i> L.			
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary.	..	..	Additional host record.
Henbane-virus (not classified)	..	..	Procter, 1944.
<b>Juncus</b> <i>novae-zelandiae</i> Hook. f.			
<i>Puccinia juncophila</i> Cke. & Mass.	..	..	Cunningham, 1945A.
<b>Linum</b> <i>marginale</i> A. Cunn.			
<i>Melampsora lini</i> (Ehrenb.) Lev.	..	..	Cunningham, 1945A.

- Linum usitatissimum** L.  
*Botrytis cinerea* Pers. .. .. Additional host record.  
*Colletotrichum linicola* Pethybr. & Laff. .. Cunningham, 1942.
- Physalis peruviana** L.  
*Entyloma australe* Speg. .. .. Cunningham, 1945c.
- Pisum sativum** L.  
*Uromyces fabae* (Pers.) de Bary. .. .. Cunningham, 1945A.
- Poa pratensis** L.  
*Claviceps purpurea* (Fr.) Tul. .. .. Neill, 1941.
- Polygonum persicaria** L.  
*Spacelotheca hydropiperis* (Schuem.) de Bary. .. Cunningham, 1945c.
- Primula polyantha** Hort.  
*Ramularia primulae* V. Thuem. .. .. Cunningham, 1944.
- Prunus armeniaca** L.  
*Verticillium dahliae* Kleb. .. .. Additional host record.
- Prunus persica** Sieb. & Zucc.  
*Xanthomonas pruni* (Smith) Dowson. .. .. Reid, 1945.  
Chlorosis (physiological) .. .. Atkinson, 1944.
- Pseudowintera axillaris** (Forst.) Dandy.  
*Uredo horopito* G. H. Cunn. .. .. Cunningham, 1945A.
- Pyrus malus** L.  
*Schizophyllum commune* Fr. .. .. Taylor & Atkinson, 1941.  
Green crinkle (physiological) .. .. Atkinson, 1946.
- Rheum raphonticum** L.  
*Puccinia rhei-undulati* (Diet) Hiratsuka. .. Cunningham, 1945A.
- Rosa** sp. cult.  
*Diplocarpon rosae* Wolf. .. .. New record.
- Rubus idaeus** L.  
*Phragmidium rubi-idaei* (DC.) Karst. .. .. Cunningham, 1945A.
- Schoenus concinnus** Hook. f.  
*Sorosporium solidum* (Berk.) McAlp. .. .. Cunningham, 1945c.
- Spergula arvensis** L.  
*Peronospora obovata* Bonord. .. .. Cunningham, 1944.
- Triticum vulgare** Vill.  
*Cercospora herpotrichoides* Fron. .. .. Saxby, 1943.  
*Claviceps purpurea* (Fr.) Tul. .. .. Neill, 1941.
- Uncinia rubra** Boott.  
*Cintractia sclerotiformis* (Cke. & Mass.) G. H. Cunn. Cunningham, 1945c.
- Veronica plebeia** R. Br.  
*Aecidium disciforme* McAlp. .. .. Cunningham, 1945A.
- Vitis vinifera** L.  
Chlorosis (physiological) .. .. Askew, 1944.
- Vittadinia australis** A. Rich.  
*Puccinia vittadiniae* McAlp. .. .. Cunningham, 1945A.
- Vulpia dertonensis** (All.) Volk.  
*Tilletia fusca* Ell. & Everh. .. .. Cunningham, 1945c.

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# THE NEW ZEALAND JOURNAL OF SCIENCE AND TECHNOLOGY

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## TOMATO - STREAK

### ITS INCIDENCE IN NEW ZEALAND AND IDENTITY WITH SINGLE VIRUS STREAK (*LYCOPERSICUM* VIRUS 1 OF SMITH, 1937—A STRAIN OF TOBACCO-MOSAIC VIRUS)

By E. E. CHAMBERLAIN, Plant Diseases Division, Department of  
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[Received for publication. 26th June, 1946]

#### Summary

(1) Tomato-streak, a virus disease causing widespread losses amongst tomatoes in New Zealand, is shown to be identical with single virus streak (*Lycopersicum* virus 1 of Smith, 1937—a strain of tobacco-mosaic virus) occurring in Britain.

(2) Incidence in individual crops is sometimes as high as 70 per cent. A survey of 54 glasshouse crops involving over 200,000 plants in six districts showed that the average infection was approximately 2.3 per cent.

(3) Under experimental conditions, transmission to tomatoes almost invariably results in the development of mosaic symptoms only.

(4) Physical properties of the virus are identical with those of tobacco-mosaic virus and, on all species tested, the host-range is similar.

(5) It may be differentiated from tobacco-mosaic by its symptoms on tobacco, on which host it causes local lesions which may or may not be followed by systemic necrosis.

In a previous paper (Chamberlain, 1940a), the author gave an account of a streak disease of tomatoes in New Zealand.\* Its symptoms were described, incidence given, and its identity discussed. In many respects it was similar to single virus streak (*Lycopersicum* virus 1 of Smith, 1937), but symptoms on tobacco and failure of the disease to produce streak symptoms on transference to tomatoes suggested that it was not identical with the British virus. Recent work has shown that a single virus streak identical with *Lycopersicum* virus 1 occurs in this country, a finding which has already been referred to in this Department's annual report for 1944-45 (Anonymous, 1945).

#### INCIDENCE

Tomato-streak has been particularly serious in field crops in the Hutt Valley during the years 1940-44 and some crops are reported to have developed practically 100 per cent. infection and become a total loss. The following reports of individual crops showing a high percentage infection

\* EDITOR'S FOOTNOTE.—Work on tomato-streak was in abeyance from 1940 to 1944 owing to the author's absence with the Armed Forces.

have been received: Auckland, 70 per cent. infection in a glasshouse of 1,500 plants in 1941, 50 per cent. infection in a glasshouse of 1,000 plants in 1945; and Hutt Valley, 25 per cent. infection in a field crop of 7,500 plants in 1945. During the 1944-45 and 1945-46 seasons, tomato specimens infected with tomato-streak were received from North Auckland, Auckland, Waikato, Wanganui, Wairarapa, Wellington, Nelson, Marlborough, North Canterbury, and Mid-Canterbury. In the past season surveys, involving a comparatively small number of crops, were made in the Auckland, Hamilton, Hutt Valley, Nelson, Blenheim, and Christchurch areas. Many crops appeared to be quite free from the disease and some showed only a small percentage infection, but in others incidence was sufficiently high to be of economic importance. Table I shows the number of crops with slight and severe infection, and Table II gives details of crops in which infection exceeded 4 per cent.

TABLE I.—INCIDENCE OF TOMATO-STREAK IN GLASSHOUSE AND FIELD

District	Date of Inspection	Number of Crops.*	Number of Crops Infected.	Number of Crops over 4 Per Cent Infection	Approximate Number of Plants.	Approximate Number infected	Average Percentage Infection.
<i>Glasshouse Crops</i>							
Auckland ..	December, 1945	9	4	3	30,900	1,290	4.2
Hamilton ..	January, 1946	3	2	2	15,000	260	1.7
Hutt Valley..	November, 1945	11	7	2	44,200	510	1.15
Nelson ..	"	8	5	2	36,000	1,220	3.4
Blenheim ..	"	6	5	3	27,000	1,040	3.75
Christchurch	"	17	6	2	51,900	390	0.75
Totals ..	..	54	29	14	205,000	4,710	2.3
<i>Field Crops</i>							
Hutt Valley..	February, 1946	6	3	1	80,000	5,120	6.4

\* When making the survey of glasshouses, all plants grown by one grower, whether in one or several houses, were considered to be one crop.

TABLE II.—INCIDENCE OF TOMATO-STREAK IN SOME TOMATO CROPS INSPECTED DURING THE 1945-46 SEASON

Date of Inspection.	Place of Occurrence.		Type of Crop	Approximate Number of Plants.	Approximate Percentage Infection.
	District.	Locality.			
Dec., 1945..	Auckland ..	St. Heliers ..	Glasshouse ..	3,100	5
" ..	" ..	Mangere ..	" ..	5,800	15
" ..	" ..	Otahuhu ..	" ..	2,600	10
Jan., 1946..	Waikato ..	Hamilton ..	" ..	4,000	5
Nov., 1945	Wellington ..	Hutt Valley	" ..	2,800	5
" ..	" ..	" ..	" ..	3,700	5
Feb., 1946..	" ..	" ..	Field ..	10,000	50
Nov., 1945	Nelson ..	Nelson City	Glasshouse ..	3,000	10
" ..	" ..	" ..	" ..	3,700	20
" ..	Marlborough ..	Blenheim ..	" ..	5,000	4
" ..	" ..	" ..	" ..	3,000	8
" ..	" ..	" ..	" ..	3,500	10
" ..	Mid-Canterbury	Christchurch	" ..	3,000	5
" ..	" ..	" ..	" ..	2,200	10

Crops inspected represent only a small fraction of those grown, but as they were selected at random the percentage of infection encountered and recorded in Table I probably gives a fairly true indication of incidence of tomato-streak throughout New Zealand.

#### SYMPTOMS ON TOMATO AND TOBACCO

##### *Tomato*

On tomato the disease is characterized by two distinct types of symptoms: (1) streak and (2) mosaic.

(1) *Streak*.—Naturally Infected Plants from Field or Glasshouse: The most characteristic symptoms are longitudinal streaks on stems and leaf petioles. They are black in colour and often continuous for the greater length of the stem. At first, streaks appear as superficial discolorations, but on cutting the stem it is found that they penetrate the cortex. In advanced stages the tissues collapse and streaks become sunken.

Dark necrotic spots develop on leaves and often extend until the leaves become brown and shrivelled, the latter symptom being hastened in many cases by severe streaking and necrosis of petioles. A slight mosaic mottling, especially of young leaves, is often but not invariably present. Usually symptoms do not appear until plants have set fruit on the bottom three or four trusses, but they have been observed on young plants shortly after setting out in the glasshouse. Plants developing streak symptoms when small remain stunted, while those developing symptoms at a later stage of growth lose much of their foliage and present a very unthrifty appearance.

The fruit often shows pronounced and very characteristic symptoms. On young fruits dark areas make their appearance, and as the fruit grows these become sunken and often crack, giving the tomatoes a scabby appearance. Dark areas also develop on more mature fruits and usually take the form of narrow bands which are sometimes arranged in arcs or circles. They also become sunken as the fruit grows, but do not crack. Such fruits have smooth, irregular, raised areas and an uneven surface.

The above symptoms are identical with those already described and illustrated by the author (Chamberlain, 1940A), except that in the previous description slight mosaic mottling of the foliage was stated to be invariably present.

Experimentally Infected Plants: In numerous attempts to transmit the disease to tomatoes from naturally infected plants only one plant developed streak symptoms. This developed typical streaking of stem and leaf petioles and necrotic spotting of leaves without any mosaic mottling of foliage. No fruits were formed, so symptoms on these could not be observed.

(2) *Mosaic*.—Symptoms are the same on naturally and experimentally infected plants and are identical with those produced by tobacco-mosaic. The leaves show a light- and dark-green mosaic mottling, are smaller than normal, and sometimes show a puckered or uneven surface. Under certain conditions leaves become narrow with pointed lobes which gives them a fern-like appearance. The plants themselves are slightly stunted, the amount of foliage is reduced, and the yield of fruit lessened. Stems may be less sturdy than those of healthy plants, but retain their normal green colour. Fruits may be small, but are not otherwise altered in appearance.

##### *Tobacco*

Tomato-streak has not been found occurring naturally on tobacco, and the following symptoms apply to plants infected under experimental conditions.

On plants inoculated by leaf-rubbing, minute dark-brown local lesions make their appearance, usually within three days. Lesions increase in size, and after seven days are  $\frac{1}{8}$  in. to  $\frac{3}{16}$  in. in diameter. They then have light-brown pin-point centres surrounded by concentric dark-brown zones (Fig. 1). Lesions reach their maximum size of approximately  $\frac{1}{4}$  in. diameter in about three weeks. Seven to ten days after inoculation dark streaks often appear on the main veins and mid-ribs of inoculated leaves (Fig. 1). Streaks may extend until the whole mid-rib is involved and the leaf turns yellow and dies.

In many cases these are the only symptoms and plants make normal growth. Sometimes, however, the disease becomes systemic. A dark streak appears usually on one side of the stem (Fig. 2), older uninoculated leaves show dark streaks extending along the mid-ribs into the larger veins, while a grey-brown necrosis occurs on veins of young leaves (Fig. 3). Growth ceases and the top of the plant curls over towards the streaked side of the stem. If plants are small when inoculated, the whole plant may collapse (Fig. 4), when death occurs within a few days. Older plants remain alive for many weeks, but die long before the controls.

#### TRANSMISSION OF TOMATO-STREAK BY ARTIFICIAL INOCULATION

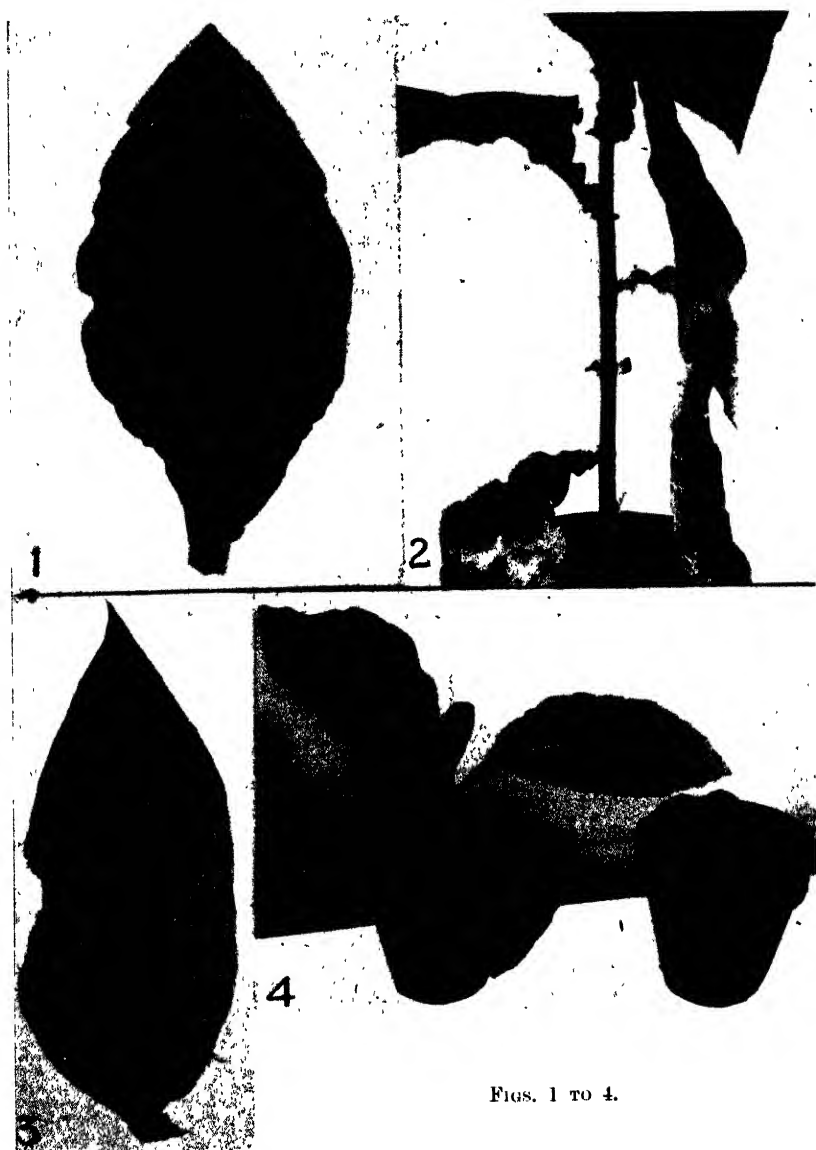
In December, 1944, diseased tomato-plant specimens were received from Wanganui. Specimens were diagnosed as being infected with tomato-streak. To verify this, four tobacco plants were inoculated by rubbing leaves with extracted juice. Within three days numerous local lesions began to appear on leaves of all four, and within twenty days two plants had developed secondary necrosis. This strain of tomato-streak was retained and used throughout the experimental work recorded herein. Many specimens of infected plants from different sources were used to inoculate tobacco, and results were the same in all cases—i.e., development of local lesions with or without secondary necrosis. Results of the inoculations are given in Table III.

TABLE III.—ARTIFICIAL TRANSMISSION OF TOMATO-STREAK TO TOBACCO

Date of Inoculation.	Source of Inoculum.				Plants inoculated.	Plants infected.	Symptoms produced.
	Host.	Locality.	Crop Type.	Symptoms.			
5/1/45	Tobacco	P.D.D.*	Glasshouse	Streak	4	4	Local lesions plus streak in 2 plants.
8/12/44	Tomato	Wanganui	"	"	4	4	Local lesions plus streak in 1 plant.
12/2/45	"	Cheviot	Field	"	4	4	Local lesions.
10/3/45	"	Hutt Valley	"	"	8	8	"
13/4/45	"	Whangarei	Glasshouse	"	1	1	"
13/4/45	"	"	"	"	1	1	"
21/4/45	"	Auckland	"	"	3	3	Local lesions plus streak in 2 plants.
4/10/45	"	Mangere	"	"	2	2	Local lesions.
*3/1/45	"	P.D.D.	"	Mosaic	4	4	Local lesions plus streak in 2 plants.
2/2/45	"	"	"	"	6	6	Local lesions.
6/4/45	"	"	"	"	1	1	"
3/5/45	"	"	"	"	12	12	"
7/9/45	"	"	"	"	4	4	"
29/9/45	"	"	"	"	3	3	"
15/10/45	"	"	"	"	2	2	"
13/11/45	"	"	"	"	6	6	"

\* P.D.D. = Plant Diseases Division, Owafraka, Auckland.

† Although tomato plants showed mosaic symptom only, the original inoculum had come from plants showing streak symptoms.



FIGS. 1 TO 4.

- Fig. 1.—Local lesions produced by tomato-streak on tobacco leaf. Note also dark streaks on veins and mid-rib.  $\times 4$ . (Photo by Miss M. J. Dahlberg.)
- Fig. 2.—Secondary symptoms of tomato-streak on tomato plant. Note dark streaks on stem and dying of inoculated leaves on which local lesions are still visible. (Photo by L. H. Wright.)
- Fig. 3.—Tomato-streak on tobacco. Secondary symptoms consisting of grey, necrotic areas on lamina of young leaf.  $\times 4$ . (Photo by Miss M. J. Dahlberg.)
- Fig. 4.—Collapse of tobacco plant inoculated with tomato-streak when small. (Photo by Miss M. J. Dahlberg.)



The development of local lesions on tobacco suggested that the virus might be identical with the British single virus streak; consequently, a series of inoculations was carried out on tomatoes. With one exception, when streak symptoms appeared, all tomato plants inoculated developed mosaic symptoms identical with those produced by tobacco-mosaic. In Table IV are given results of these inoculations.

TABLE IV.—ARTIFICIAL TRANSMISSION OF TOMATO-STREAK TO TOMATO

Date of Inoculation.	Source of Inoculum.				Plants inoculated.	Plants infected.	Symptoms produced.
	Host.	Locality.	Crop Type.	Symptoms.			
19/12/44 ..	Tobacco	P.D.D.*	Glasshouse ..	Streak ..	5	5	Mosaic.
5/1/45 ..	"	"	"	"	4	4	"
20/2/45 ..	"	"	"	"	4	4	"
13/4/45 ..	Tomato	Whangarei	"	"	10	10	"
20/6/45 ..	"	P.D.D.	"	"	6	6	"
31/10/45 ..	"	Otahuhu	"	"	2	2	"
3/1/45 ..	"	P.D.D.	"	Mosaic	4	4	"
24/1/45 ..	"	"	"	"	4	4	"
7/3/45 ..	"	"	"	"	4	4	"
6/4/45 ..	"	"	"	"	4	4	"
13/4/45 ..	"	Whangarei	"	"	15	15	"
11/5/45 ..	"	P.D.D.	"	"	6	6	"
1/6/45 ..	"	"	"	"	6	6	5 " plants mosaic; plant streak.
20/6/45 ..	"	"	"	Streak	6	6	Mosaic.
12/7/45 ..	"	"	"	Mosaic	6	6	"
8/8/45 ..	"	"	"	"	3	3	"
7/9/45 ..	"	"	"	"	6	6	"
20/9/45 ..	"	"	"	"	3	3	"
15/10/45 ..	"	"	"	"	14	14	"

#### HOST-RANGE OF TOMATO-STREAK AND COMPARISON OF SYMPTOMS WITH THOSE OF TOBACCO-MOSAIC

To ascertain if symptoms produced by tomato-streak differed from those of tobacco-mosaic, inoculations were made with the two viruses on a number of solanaceous species. Results are given in Table V.

TABLE V.—HOST-RANGE OF TOMATO-STREAK AND COMPARISON WITH TOBACCO-MOSAIC

Plant inoculated.	Date of Inoculation.	Source of Inoculum.		Plants inoculated.	Plants infected.	Symptoms.
		Virus.	Host.			
Tomato ( <i>Lycopersicon esculentum</i> )	7/3/45.	Tomato-streak	Tomato	4	4	Mosaic
Tobacco ( <i>Nicotiana tabacum</i> )	"	Tobacco-mosaic	Tobacco	4	4	"
"	"	Tomato-streak	Tomato	3	3	Local lesions.
"	"	Tobacco-mosaic	Tobacco	3	3	Mosaic.
Devil's thorn-apple ( <i>Datura stramonium</i> )	"	Tomato-streak	Tomato	4	4	Local lesions.
"	"	Tobacco-mosaic	Tobacco	4	4	Local lesions plus stem streak in 1 plant.
"	9/11/45	Tomato-streak	Tomato	4	4	Local lesions.
"	"	Tobacco-mosaic	Tobacco	4	4	Local lesions plus stem streak in 2 plants.
Turkistan tobacco ( <i>Nicotiana glauca</i> )	9/3/45	Tomato-streak	Tomato	4	4	Mosaic and necrosis.
"	"	Tobacco-mosaic	Tobacco	4	4	"
<i>Nicotiana glauca</i>	20/3/45	Tomato-streak	Tomato	4	4	"
"	"	Tobacco-mosaic	Tobacco	4	4	"
"	6/4/45.	Tomato-streak	Tomato	1	1	Local lesions, stem streak, vein-clearing, and necrosis.
"	"	Tobacco-mosaic	Tobacco	1	1	"
Cape gooseberry ( <i>Physalis peruviana</i> )	12/3/45	Tomato-streak	Tomato	4	4	Mosaic.
"	"	Tobacco-mosaic	Tobacco	4	4	"
Egg-plant ( <i>Solanum melongena</i> )	27/4/45	Tomato-streak	Tomato	4	4	Local lesions plus necrosis in 2 plants.
"	"	Tobacco-mosaic	Tobacco	4	4	Local lesions plus necrosis in 1 plant.
Tree-tomato ( <i>Cyphomandra beracea</i> )	14/2/46	Tomato-streak	Tomato	3	0	"
"	"	Tobacco-mosaic	Tobacco	2	0	"

These indicate that both viruses have a similar host-range and that only on tobacco are symptoms markedly different. On devil's thorn-apple, streaks developed on stems of three out of eight plants inoculated with tobacco-mosaic, while none appeared on eight plants inoculated with tomato-streak. It is doubtful if this is a significant difference, since Miss Jarrett (1930) found that streaks were formed on stems of devil's thorn-apple plants inoculated with tomato-streak.

#### PHYSICAL PROPERTIES OF TOMATO-STREAK

To verify the identity of the New Zealand tomato-streak, its physical properties were ascertained.

##### *Dilution End-point*

Three tests were made to determine the greatest dilution at which infection could be brought about. Inoculum was obtained from experimentally infected tomato plants showing mosaic symptoms. Using the leaf-rubbing method inoculations were made on "Warne" tobacco plants.

Results, given in Table VI, show that in the different experiments the dilution end-point ranged from 1-500,000 to 1-2,500,000. It is therefore of the same order as that of tobacco-mosaic.

TABLE VI.—TOMATO-STREAK: DILUTION END-POINT

(All inoculations carried out on tobacco plants using juice from infected tomato plants)

Strength of Inoculum				Plants inoculated	Plants infected	Local Lesions
<i>Experiment carried out 6th March, 1945</i>						
1-	1	..	..	4	4	Very numerous.
1-	100	..	..	4	4	"
1-	1,000	..	..	4	4	"
1-	10,000	..	..	4	4	Numerous.
1-	50,000	..	..	4	4	22
1-	100,000	..	..	4	3	20
1-	500,000	..	..	4	3	7
1-	1,000,000	..	..	4	0	0
<i>Experiment carried out 20th March, 1945</i>						
1-	1	..	..	4	4	Very numerous.
1-	10,000	..	..	4	4	Numerous.
1-	100,000	..	..	4	4	8
1-	500,000	..	..	4	1	3
1-	1,000,000	..	..	4	0	0
1-	2,500,000	..	..	4	0	0
<i>Experiment carried out 1st June, 1945</i>						
1-	1	..	..	3	3	Very numerous.
1-	10,000	..	..	3	3	210
1-	100,000	..	..	6	6	75
1-	500,000	..	..	6	5	23
1-	1,000,000	..	..	6	5	15
1-	2,500,000	..	..	6	5	8

##### *Thermal Death-point*

Thermal death-point determinations were made by a method described by Chamberlain (1937). In two experiments, inoculations were made by the leaf-rubbing method on "Warne" tobacco plants with inoculum from experimentally infected tomato plants showing mosaic symptoms.

Results given in Table VII show that the thermal death-point is 90° c., being the same as that of tobacco-mosaic virus.

TABLE VII.—TOMATO-STREAK: THERMAL DEATH-POINT  
(Inoculations carried out on tobacco plants using juice from infected tomato plants)

Temperature, in Degrees Centigrade	Plants inoculated.	Plants infected.	Local Lesions.
<i>Experiment carried out 28th February, 1945</i>			
24	4	4	Very numerous.
75	4	4	"
80	4	4	"
84	4	4	Numerous.
86	4	4	"
88	4	3	4
90	4	0	0
92	4	0	0
<i>Experiment carried out 4th May, 1945</i>			
18	4	4	Very numerous.
84	4	4	"
86	4	4	Numerous.
88	4	3	11
90	4	1	1
92	4	0	0

*Longevity in vitro and in Dried Leaf*

Experiments were commenced in February, 1945, to ascertain longevity of the virus *in vitro* and in dried leaf. On 20th February, 1945, leaves taken from an experimentally infected tomato plant which showed mosaic symptoms were air-dried and stored at room temperatures of 10° c. to 30° c. Juice extracted from leaves of the same plant was stored in a test-tube in an incubator at 23° c. From time to time the samples were tested for presence of tomato-streak by leaf-rubbing inoculations on "Warne" tobacco plants. The last test was made on 18th June, 1946, the virus being still viable in both samples, showing that it had survived in extracted juice and dried leaf for sixteen months. The results indicate that the virus has a longevity similar to that of tobacco-mosaic virus.

IDENTITY OF NEW ZEALAND TOMATO-STREAK

As has been previously indicated (Chamberlain, 1940A, 1940B), streak symptoms in tomatoes may be caused by a combination of tobacco-mosaic with any one of a number of other viruses or by a single virus. The disease discussed in this paper is of the latter type.

Miss Jarrett (1930) demonstrated that the single virus streak occurring in Britain had physical properties identical with those of tobacco-mosaic. She also showed that both viruses possessed the same host-range and produced similar symptoms on all hosts except tomatoes. Ainsworth (1933) also demonstrated that it had the same physical properties and host-range as tobacco-mosaic, but found that the two viruses could be separated by their differing symptoms on tobacco and egg-plant. Tobacco-mosaic produced a mosaic mottling of foliage in both tobacco and egg-plant, while streak produced in tobacco local lesions sometimes followed by systemic necrosis and in egg-plant local lesions followed by necrosis and mild mosaic.

The tomato-streak virus discussed previously (Chamberlain 1940A) agreed very closely with that described by Miss Jarrett, the only difference being that whereas Miss Jarrett found that on artificial inoculation the disease produced streak symptoms on some tomato plants and mosaic on others, the author found that all plants developed mosaic. The virus described herein agrees more closely with Ainsworth's description, as it has the same physical properties and host-range as tobacco-mosaic and produces identical symptoms on all hosts tested other than tobacco and occasionally tomato. It differs from the single virus streak described by Ainsworth only in that it cannot be differentiated from tobacco-mosaic by symptoms on egg-plant. However, symptoms of tobacco-mosaic on egg-plant are not a reliable diagnostic character, as they appear to be variable. Thus tobacco-mosaic symptoms on egg-plant have been variously reported as follows:—

Johnson (1927)	..	..	Necrosis.
Ainsworth (1933)	..	..	Slight mottle and stunting.
Chamberlain (1937)	..	..	Some plants necrosis and others slight mottling.
Chamberlain (present paper)			Local lesions followed by necrosis.

It would therefore appear that the British and New Zealand single virus streaks are identical.

Naming the virus presents difficulties. Before it was realized that more than one virus was involved in producing streak diseases of tomatoes, the terms "stripe," "black stripe," "winter blight," and "severe mosaic" appear to have been used more or less indiscriminately. Miss Jarrett (1930) applied the term "streak" to the disease caused by the single virus, but also referred to it as "glasshouse streak." The name "glasshouse streak" was adopted by Ainsworth (1933), but later Ainsworth *et al.* (1934) referred to it as "single virus streak" and named the virus "tomato-streak virus No. 1." Berkeley (1936) suggested that "tomato-streak virus No. 1" was a strain of tobacco virus 1, but Smith (1937) considered that it was a distinct virus and classified it as "*Lycopersicum* virus 1." Since then he (Smith, 1945) has referred to it as a strain of tobacco-mosaic virus.

Although the physical properties, host-range, and symptoms suggest that the virus is probably a strain of tobacco-mosaic, it is still necessary to differentiate it from the type virus. Consequently, it is proposed to use the name "*Lycopersicum* virus 1 of Smith (1937)—a strain of tobacco-mosaic virus."

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## A LIST OF PLANT VIRUS DISEASES IN NEW ZEALAND

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### INTRODUCTION

OWING to the lack of published information and to the wide host range of some of the plant virus diseases occurring in New Zealand, difficulty is experienced in recognizing and naming them. This list has been prepared with a view to assisting in their recognition. It comprises records of thirty-one plant virus diseases which have been found in the Dominion, together with sixty-six host plants found to be susceptible under field conditions.

Diseases have been listed under their common names, following as closely as possible those used in *The Review of Applied Mycology* (24, 513-56 (1945)). Where possible, viruses have been named according to the classification adopted by K. M. Smith in "A Text-book of Plant Viruses" (1937). A number have not been fully investigated, but are obviously the same as those described in Dr. Smith's book, and have been classified accordingly. Where their identity is doubtful, viruses have been listed as "Not classified."

The list is necessarily incomplete, since there are several diseases at present under investigation which may eventually prove to be of a virus nature.

The host list may be used as a reference to diseases with which are given the virus name, host range, and bibliography of the more important papers published in New Zealand.

### PLANT VIRUS DISEASES PRESENT IN NEW ZEALAND

Disease.	Virus	Hosts.*	Reference.
Bean-mosaic ..	<i>Phaseolus</i> virus 1 ..	Dwarf bean, runner bean	Chamberlain, 1939a.
Beet-mosaic (syn. sugar-beet mosaic)	<i>Beta</i> virus 1 ..	Sugar-beet, mangel	Cunningham, 1941 ; Matthews, 1945.
Cucumber-mosaic (syn. tomato narrow-leaf)	<i>Cucumis</i> virus 1 ..	Cucumber, marrow, pumpkin, rock-melon, tomato, polyanthus, viola, pansy	Chamberlain, 1934b, 1939b.
Cucumber-mosaic (syn. lily-mosaic)	Lily-mosaic strain of <i>Cucumis</i> virus 1	Lily .. ..	Brien, 1939.
Daphne-mosaic ..	Not classified ..	Daphne ..	Chamberlain and Matthews, 1941.
Dock-mosaic ..	Not classified ..	Dock, fiddle dock, curled dock	New record.
Fig-mosaic ..	<i>Ficus</i> virus 1 ..	Fig .. ..	Li and Procter, 1944.
Henbane-mosaic ..	Not classified ..	Henbane ..	Procter, 1944.
Iris-mosaic ..	<i>Iris</i> virus 1 ..	Bulbous irises ..	New record.
Lettuce-mosaic ..	Not classified ..	Lettuce ..	New record.
Narcissus-mosaic ..	Not classified ..	Narcissus ..	New record.
Onion yellow-dwarf..	<i>Allium</i> virus 1 ..	Onion .. ..	Chamberlain and Baylis, 1939.
Passion-fruit woodiness	<i>Passiflora</i> virus 1 ..	Passion-fruit ..	Cunningham, 1939.
Pea-mosaic (syn. soreshin of blue lupins)	<i>Pisum</i> virus 2 ..	Garden pea, field pea, sweet pea, broad bean, blue lupin, red clover, subterranean clover, white lupin, yellow lupin, <i>Lupinus mutabilis</i>	Niell <i>et al.</i> , 1934 ; Chamberlain, 1935, 1938, 1937, 1939c, 1940.

PLANT VIRUS DISEASES PRESENT IN NEW ZEALAND—*continued*

Disease.	Virus.	Hosts.*	Reference.
Pea-streak ..	<i>Pisum</i> virus 3 ..	Garden pea ..	Chamberlain, 1939.
Potato aucuba-mosaic	<i>Solanum</i> virus 9 ..	Potato .. ..	Cunningham, 1929.
Potato-crinkle ..	Not classified ..	Potato .. ..	Hadfield and Claridge, 1929.
Potato leaf-roll ..	<i>Solanum</i> virus 14	Potato .. ..	Cottier, 1931; Cunningham, 1929; Hadfield and Claridge, 1929; Hadfield <i>et al.</i> , 1931.
Potato virus X (syn. potato-mosaic)	<i>Solanum</i> virus 1 ..	Potato .. ..	Cunningham, 1929; Hadfield and Claridge, 1929; Hadfield <i>et al.</i> , 1931.
Potato masked virus	Probably a strain of <i>Solanum</i> virus 1	Potato .. ..	Chamberlain, 1940c.
Potato virus Y (syn. potato stipple-streak)	<i>Solanum</i> virus 2 ..	Potato .. ..	Cunningham, 1929; Hadfield and Claridge, 1929; Hadfield <i>et al.</i> , 1931.
Rhubarb-mosaic ..	Not classified	Rhubarb ..	New record.
Strawberry yellow-edge	<i>Fragaria</i> virus 1 ..	Strawberry ..	Chamberlain, 1934A; Woodhead and Chamberlain, 1940.
Tobacco-mosaic (syn. tomato-mosaic)	<i>Nicotiana</i> virus 1 ..	Tobacco, Turkestan tobacco, tomato, Cape gooseberry, black nightshade, chilli	Chamberlain, 1934, 1937A, 1937B; Chamberlain and Clark, 1937; Thomson, 1945.
Tobacco-mosaic (syn. tomato-streak, tomato-stripe, tomato black-stripe)	Tomato-streak strain of <i>Nicotiana</i> virus 1	Tomato ..	Chamberlain, 1940A, 1946; Taylor, W. H. 1922, 1923.
Tobacco vein-banding necrosis	Not classified ..	Tobacco ..	Chamberlain, 1940B.
Tomato-necrosis ..	Not classified ..	Tomato ..	Chamberlain, 1940E, 1940D.
Tomato spotted-wilt	<i>Lycopersicum</i> virus 3	Tomato, tobacco, lettuce, Iceland poppy, anemone, aster, calendula, dahlia, nasturtium, zinnia, blacknightshade, chrysanthemum, garden pea†, hennip†, calla†, flowering lupin†, devil's thorn-apple†, <i>Datura metel</i> †	Chamberlain and Taylor, 1936, 1938; Taylor and Chamberlain, 1937.
Tomato-streak mixed virus (syn. tomato severe mosaic, tomato severe-streak)	<i>Nicotiana</i> virus 1 plus tomato-necrosis virus	Tomato ..	Chamberlain, 1934, 1940E, 1940D.
Tree-tomato mosaic	Not classified ..	Tree tomato ..	New record.
Tulip-breaking ..	<i>Tulipa</i> virus 1 ..	Tulip .. ..	Brien, 1939.
Turnip-mosaic ..	<i>Brassica</i> virus 1 ..	Turnip, swede, rape, white mustard, stock, wallflower	Chamberlain, 1936A, 1939D.
Violet-mosaic ..	Not classified ..	Violet .. ..	New record.

\* Only hosts found to be susceptible under field conditions are listed.

† New host records.

## NATURAL HOSTS OF PLANT VIRUS DISEASES IN NEW ZEALAND

Host.	Disease.
Anemone ( <i>Anemone coronaria</i> L.)* .. ..	Tomato spotted-wilt.
Aster ( <i>Callistephus chinensis</i> Nees.) .. ..	Tomato spotted-wilt.
Bean—	
Dwarf ( <i>Phaseolus vulgaris</i> L.) .. ..	Bean-mosaic.
Runner ( <i>Phaseolus multiflorus</i> Willd.) .. ..	Bean-mosaic.
Broad ( <i>Vicia faba</i> L.) .. ..	Pea-mosaic.
Black nightshade ( <i>Solanum nigrum</i> L.) .. ..	Tobacco-mosaic, tomato spotted-wilt.
Calendula ( <i>Calendula officinalis</i> L.) .. ..	Tomato spotted-wilt.
Calla ( <i>Zantedeschia</i> sp. cult.) .. ..	Tomato spotted-wilt.
Cape gooseberry ( <i>Physalis peruviana</i> L.) .. ..	Tobacco-mosaic.
Chilli ( <i>Capsicum annum</i> L.) .. ..	Tobacco-mosaic.
Chrysanthemum ( <i>Chrysanthemum</i> sp. cult.) .. ..	Tomato spotted-wilt.
Clover—	
Red ( <i>Trifolium pratense</i> L.) .. ..	Pea-mosaic.
Subterranean ( <i>Trifolium subterraneum</i> L.) .. ..	Pea-mosaic.
Cucumber ( <i>Cucumis sativus</i> L.) .. ..	Cucumber-mosaic.
Dahlia ( <i>Dahlia</i> sp. cult.) .. ..	Tomato spotted-wilt.
Daphne ( <i>Daphne odora</i> Thunb.) .. ..	Daphne-mosaic.
<i>Datura metel</i> L. .. ..	Tomato spotted-wilt.
Devil's thorn-apple ( <i>Datura stramonium</i> L.) .. ..	Tomato spotted-wilt.
Dock ( <i>Rumex obtusifolius</i> L.) .. ..	Dock-mosaic.
Fiddle ( <i>Rumex pulcher</i> L.) .. ..	Dock-mosaic.
Curled ( <i>Rumex crispus</i> L.) .. ..	Dock-mosaic.
Fig ( <i>Ficus carica</i> L.) .. ..	Fig-mosaic.
Henbane ( <i>Hyoscyamus niger</i> L.) .. ..	Henbane-mosaic, tomato spotted-wilt.
Iceland poppy ( <i>Papaver nudicaule</i> L.) .. ..	Tomato spotted-wilt.
Iris, bulbous ( <i>Iris longitana</i> Boiss.) .. ..	Iris-mosaic.
Dutch ( <i>Iris xiphium</i> L.) .. ..	Iris-mosaic.
Kumi kumi ( <i>Cucurbita pepo</i> L.) .. ..	Cucumber-mosaic.
Lily ( <i>Lilium longiflorum</i> Thunb.) .. ..	Cucumber-mosaic (hly-mosaic strain).
( <i>Lilium</i> sp. cult.) .. ..	Cucumber-mosaic (hly-mosaic strain).
Lettuce ( <i>Lactuca sativa</i> L.) .. ..	Tomato spotted-wilt, lettuce-mosaic.
Lupin—	
Blue ( <i>Lupinus angustifolius</i> L.) .. ..	Pea-mosaic.
Flowering ( <i>Lupinus polyphyllus</i> Indl.) .. ..	Tomato spotted-wilt.
White ( <i>Lupinus albus</i> L.) .. ..	Pea-mosaic.
Yellow ( <i>Lupinus luteus</i> L.) .. ..	Pea-mosaic.
<i>Lupinus mutabilis</i> L. .. ..	Pea-mosaic.
Mangel ( <i>Beta vulgaris</i> L.) .. ..	Beet-mosaic.
Marrow ( <i>Cucurbita pepo</i> L.) .. ..	Cucumber-mosaic.
Mustard, white ( <i>Sinapis alba</i> L.) .. ..	Turnip-mosaic.
Narcissus ( <i>Narcissus</i> sp. cult.) .. ..	Narcissus-mosaic.
Nasturtium ( <i>Tropaeolum majus</i> L.) .. ..	Tomato spotted-wilt.
Onion ( <i>Allium cepa</i> L.) .. ..	Onion yellow-dwarf.
Pansy ( <i>Viola tricolor</i> L.) .. ..	Cucumber-mosaic.
Passion-fruit ( <i>Passiflora edulis</i> Simms.) .. ..	Passion-fruit woodiness.
Pea—	
Garden ( <i>Pisum sativum</i> L.) .. ..	Pea-mosaic, pea-streak, tomato spotted-wilt.
Field ( <i>Pisum sativum</i> L. var. <i>arvense</i> Poir.) .. ..	Pea mosaic.
Sweet ( <i>Lathrus odoratus</i> L.) .. ..	Pea-mosaic.
Polyanthus ( <i>Primula polyantha</i> Hort.) .. ..	Cucumber-mosaic.
Potato ( <i>Solanum tuberosum</i> L.) .. ..	Potato aucuba-mosaic, potato crinkle, potato leaf-roll, potato masked virus, potato virus X, potato virus Y.
Pumpkin ( <i>Cucurbita pepo</i> L.) .. ..	Cucumber-mosaic.
Rape ( <i>Brassica napus</i> L.) .. ..	Turnip-mosaic.
Rhubarb ( <i>Rheum raphanticum</i> L.) .. ..	Rhubarb-mosaic.
Rock-melon ( <i>Cucumis melo</i> L. var. <i>cantalupinus</i> Naud.) .. ..	Cucumber-mosaic.
Stock ( <i>Matthiola incana</i> R. Br.) .. ..	Turnip-mosaic.
Strawberry ( <i>Fragaria</i> sp. cult.) .. ..	Strawberry yellow-edge.

\* Recorded by Chamberlain and Taylor, 1938, as *Anemone hortensis*.

## NATURAL HOSTS OF PLANT VIRUS DISEASES IN NEW ZEALAND—continued

Host.	Disease.
Sugar-beet ( <i>Beta vulgaris</i> L.) .. ..	Beet-mosaic.
Swede ( <i>Brassica napo-brassica</i> DC.) .. ..	Turnip-mosaic.
Tobacco ( <i>Nicotiana tabacum</i> L.) .. ..	Tobacco-mosaic, tomato spotted-wilt, tobacco vein-banding necrosis.
Turkestan ( <i>Nicotiana rustica</i> L.) .. ..	Tobacco-mosaic.
Tomato ( <i>Lycopersicum esculentum</i> Mill.) .. ..	Tobacco-mosaic, tomato spotted-wilt, cucumber-mosaic, tomato-streak mixed virus, tomato-necrosis, tobacco-mosaic (tomato-streak strain).
Tree tomato ( <i>Cyphomandra belacea</i> Sendt.) .. ..	Tree-tomato mosaic.
Tulip ( <i>Tulipa</i> sp. cult.) .. ..	Tulip-breaking.
Turnip ( <i>Brassica rapa</i> L.) .. ..	Turnip-mosaic.
Viola ( <i>Viola tricolor</i> L.) .. ..	Cucumber-mosaic.
Violet ( <i>Viola odorata</i> L.) .. ..	Violet-mosaic.
Wallflower ( <i>Cheiranthus cheiri</i> L.) .. ..	Turnip-mosaic.
Zinnia ( <i>Zinnia elegans</i> Jacq.) .. ..	Tomato spotted-wilt.

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## NOTES ON CLASSIFICATION OF THE POLYPORACEÆ

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### Summary

- (1) It is shown that delimitation of genera and species of the Polyporaceæ may be implemented by use of such microscopic features as hyphal systems, hyphal series, hyphal types, hyphal colour, basidial types, and context inclusions.
- (2) Three hyphal systems are present in the family—monomitic, dimitic, and trimitic.
- (3) They may be composed of three hyphal series—generative, skeletal, and binding.
- (4) "Bovista" and "long" hyphal types are defined.
- (5) Three basidial types are described—"meruloid," "honeycomb," and "clavate."

UNDER the family Polyporaceæ are placed fungi which possess one feature in common. The hymenium is developed upon the inner surface of pores which penetrate into the context of the hymenophore, or fruit-body. Pores may enter deeply into the context, or be shallow—1 mm. or less in depth—and, in extreme cases, as in species of *Merulius*, they appear as little more than depressions upon the surface of the hymenophore. The term "pore" is, however, used somewhat loosely, for in different genera pores may be orbicular and tubelike, favoid, labyrinthiform, or (in *Lenzites*) narrow cavities bounded by gill-like lamellæ. The hymenophore of different species may be carried upon a central definite stem like that of an agaric, be applanate and attached laterally by a narrow or broad base, ungulate, or resupinate upon the substratum. Its surface may be smooth, hairy, provided with a thin cuticle, or covered with a thick, hardened, sometimes laccate crust. A few species are parasitic upon living trees, but most are saprophytes, growing upon dead wood or rich vegetable debris upon the ground.

Genera of the Boletaceæ and Hydnaceæ are excluded—the former since the hymenium lines well-defined separable tubes; the latter because the hymenium is carried upon the exterior of spines or teeth.

According to the International Rules of Botanical Nomenclature, the starting-point of nomenclature in the family is Fries' *Systema Mycologicum*, Vol. 1, 1821. In this work Fries recognized but three genera—*Merulius* (p. 326), *Daedalea* (p. 331), and *Polyporus* (p. 341). Under *Merulius* were placed species with shallow pores formed by coalescence of veins or folds of the hymenium carried upon a thin resupinate hymenophore. *Daedalea* was characterized by the fact that the hymenium covered gill-like dissepiments arranged to form poroid, labyrinthiform, or lamellate cavities, the hymenophore being woody or coriaceous and possessing a definite margin. The genus was subdivided into three tribes—*Stipitatae*, containing species with a definite stem; *Dimidiatae*, for species which were attached laterally by a lateral base; and *Resupinatus*, for species with a resupinate hymenophore. *Polyporus* contained species with a poroid hymenium and fleshy or coriaceous hymenophore. Three subgenera were recognized—*Favolus* with large, 4 to 6 sided pores; *Microporus* with orbicular and minute pores; and *Polysticta* with shallow pores. *Microporus* was further divided into five tribes—*Mesopus*, with a central stem; *Pleuropus*, with a lateral stem; *Merisma* contained species with much branched stems; *Agus*, with a sessile dimidiate hymenophore; and *Resupinatus*, with a resupinate hymenophore.

In "Genera Hymenomycetum" (1836) Fries established several additional genera. *Lenzites* (p. 10) was separated from *Daedalea* to contain species with gill-like dissepiments. He placed it with the agarics, where it remained until Hennings (1898) showed that it was a member of the Polyporaceæ. *Trametes* was erected to contain species with orbicular pores formerly placed under *Daedalea*. Fries separated the genus from *Polyporus* on the ground that tissues of the context penetrated unchanged between the pores and were concolorous with the latter, whereas in *Polyporus* pore tissues were held to be of different colour. As these differences were too indefinite to enable separation to be made between *Trametes* and *Polyporus*, Karsten (1889, p. 287) limited *Trametes* to species in which pores penetrated different depths into the context, forming an irregular layer. Fries also recognized as valid the genera *Favolus* Beauv., *Hexagonia* Poll., and *Cyclomyces* Kze. & Fr., separating them from *Polyporus* because of the favoid, 4 to 6 sided pores and concentric lamellæ respectively. *Laschia* Fr. (1830, p. 533) was held to differ from *Polyporus* in its gelatinous hymenophore.

Fries later (1851) published a more concise delimitation of genera he proposed to recognize. *Polyporus* was subdivided into three subgenera—*Eupolyporus*, for species with an annual coriaceous hymenophore; *Fomes*, for those with a woody hymenophore and pores in strata; and *Poria*, for resupinate species. He separated *Polystictus* from *Polyporus* to contain species with a thin and coriaceous hymenophore, fibrous cuticle, and different arrangement of hyphæ in the tramal walls. *Polystictus* was abandoned in his last major work (1874).

In "Hymenomycetes Europaei" (1874) Fries recognized nine genera in all—*Lenzites* (p. 492), *Polyporus* (p. 522), *Trametes* (p. 581), *Daedalea* (p. 586), *Cyclomyces* (p. 589), *Hexagonia* (p. 589), *Favolus* (p. 590), *Laschia* (p. 591), and *Merulius* (p. 591). All were erected upon macroscopic features previously discussed.

With certain modifications most were accepted by earlier mycologists who worked over the family. A few, however, recognizing their inadequacy and the variable nature of the characters upon which species were based, attempted to modify or improve the classification finally adopted by Fries.

Gillet (1878) subdivided *Polyporus* into *Fomes* (p. 682), *Merisma* (p. 688), and *Physisporus* (p. 694). Under the first he placed species with pores in strata, the second those with compound stems, and under the third, resupinate species placed by Fries under his subgenus *Poria*.

Quelet (1886) raised to generic rank eleven of the sections into which Fries (1851) had divided *Polyporus*, according to the presence or absence of a stem and type of surface of the hymenophore. Genera recognized were *Caloporus* (p. 164), *Leucoporus* (p. 165), *Pelloporus* (p. 166), *Ceriporus* (p. 167), *Cladomeris* (p. 167), *Placodes* (p. 170), *Phellinus* (p. 172), *Inodermis* (p. 173), *Leptoporus* (p. 175), *Coriolus* (p. 175), and *Poria* (p. 177).

Karsten, in a series of papers published between 1879 and 1889, subdivided Fries' genera into many. Divisions were made on characters accepted as generic by Fries with such others as colour and texture of the context, colour of spores, presence or absence of setæ, depth and shape of the pores. *Polyporus* (1879) was subdivided into *Polyporellus* (p. 37), *Bjerkandera* (p. 38), *Ischnoderma* (p. 38), *Hansenia* (p. 39), *Inonotus* (p. 39), *Inoderma* (p. 39), and *Antrodia* (p. 40). *Fomes* (1881) was split into *Ganoderma* (p. 17), *Piptoporus* (p. 17), *Polypilus* (p. 17), *Postia* (p. 17), *Tyromyces* (p. 17), *Hapalopilus* (p. 18), *Pycnoporus* (p. 18), and *Fomitopsis* (p. 18). Karsten

later (1882) erected *Meripilus* (p. 33), *Gloeophyllum* (p. 79), and *Xylophilus* (p. 69); and, in 1883, *Phyllocladia* (p. 163). Finally (1889) he established *Onnia* (p. 326), *Elfvigia* (p. 333), and *Lenzitina* (p. 337).

Patouillard (1887) used as generic characters features employed by Fries with, in addition, differences in surface, structure and form of the hymenophore, spore colour, form and presence or absence of a stem. He placed certain sections of Fries' genera under *Melanopus* (p. 80), *Spongipellis* (p. 84), *Phaeolus* (p. 86), *Xanthochrous* (p. 95), *Funalia* (p. 95), and *Ungulina* (p. 102).

A modification of Patouillard's later classification (1900) was used by Carleton Rea (1922). Two families were erected because of supposed differences in structure of the hymenium. Genera of the Polyporaceæ, containing *Polyporus*, *Sistotrema*, *Fomes*, *Ganoderma*, and *Poria*, were held to possess a hymenial layer distinct from the tissues of the context. Those of the Polystictaceæ, containing *Polystictus*, *Irpex*, *Lenzites*, *Trametes*, and *Daedalea*, were said to possess a hymenial layer homogeneous with tissues of the context. A third family, Meruliaceæ, was erected for *Merulius* and four other genera.

The most complex system yet to appear is that of Murrill (1907-1908). No less than 78 genera were recognized, of which 39 were erected by himself, 29 being monotypic. They were placed under four tribes. The Poriceæ contained genera with a resupinate hymenophore, the Polyporeæ those with stipitate or dimidiate annual hymenophores, the Fomiticeæ genera with a perennial stratose hymenophore, and the Daedaleæ genera with a furrowed hymenium. Colour of context and spores, depth colour and arrangement of pores, presence or absence of a stem, consistency of the context and surface were used in generic segregation.

Adeline Ames (1913) made a detailed survey of morphological features used as generic characters by previous workers. She concluded that broader relationships were best shown by consistency of the hymenophore and characters of the context. Using these as a basis, she divided species into groups which were further separated into genera on account of surface modifications and form of the hymenophore, configuration of the hymenium and spore characters. Sixteen genera were recognized and described.

Such diversity of treatment by different specialists shows that the Friesian system is inadequate and does not provide a suitable grouping for species of the family. Especially is this the case when attempts are made to classify a large number of plants, particularly species from tropical and subtropical areas. The reason lies in the fact that generic characters were based on variable macroscopic features, such as presence or absence and type of stem, nature of context and cuticle, and, particularly, configuration of the hymenial surface. That these are variable may be shown by a few specific examples.

In different taxonomic papers species with labyrinthiform, lamellate, or poroid hymenia may be placed under *Daedalea* or *Trametes*; plants with or without stem or stratose pores under *Fomes*; those with poroid or dædaloid pores in one or several strata under *Poria*. Under *Lenzites* may be grouped plants with either lamellar or dædaloid type hymenium; sometimes both conditions may occur in the same specimen. Under *Polyporus* and *Poria* may be found species which more properly belong to *Merulius*. *Polystictus* cannot be separated from *Polyporus* on any constant character of those used in the Friesian treatments.

The position is even more confusing in certain widely distributed species, where different specimens exhibit characters upon which they could be (and often have been) placed under one of several genera.

(1) *Polyporus hirsutus* possesses a hymenophore which may be thin and coriaceous, with pores in a single layer, orbicular, and inserted to the same depth in the context. Specimens from tropical and subtropical areas may be thick and woody; pores are sometimes in strata, and may be poroid or labyrinthiform, sometimes exhibiting both conditions in the same plant. Pores may also be inserted at different depths in the context. Resupinate specimens are not uncommon. The species could therefore be placed under any one of six genera—*Polystictus*, *Polyporus*, *Fomes*, *Daedalea*, *Trametes*, and *Poria*.

(2) *Trametes cinnabarina* is a red species common through the temperate and subtropic regions of the world. Usually the hymenophore is coriaceous, thin, with one pore layer inserted in the context to a constant depth (*Polystictus*). Plants at hand also possess a thick and floccose hymenophore (*Polyporus*), a resupinate hymenophore (*Poria*), pores inserted at different depths (*Trametes*), or pores in strata (*Fomes*).

(3) *Trametes pini* was erected upon forms with a single layer of orbicular pores inserted at different depths in the context. Specimens may also exhibit labyrinthiform pores (*Daedalea*), poroid or labyrinthiform pores in strata (*Fomes*), or a resupinate hymenophore (*Poria*).

It is therefore evident that some better system of delimiting genera is required. As has been shown, several workers have attempted to provide this, but with indifferent success since they employed variable characters used by Fries, alone or implemented by others equally, or even more, variable. More recently several workers have attempted the use of microscopic or cultural features to implement those of the Friesian system.

Overholts (1929), by applying the microscopic technique elaborated by E. A. Burt in his classical studies of the Thelephoraceæ, showed that specific diagnostic aids lay to hand in a study of sterile bodies present in context and hymenium, certain hyphal characters, differences in the context and the like.

Baxter (1933-1945), in a long series of papers, used as differential characters cultural features observed when species were grown upon wood blocks in various culture media. His studies were confined mainly to resupinate species of the family.

Corner (1932A, 1932B) covered original ground by making detailed studies of microscopic structures. His work has shown that valuable aids to classification lie in the hyphal series and hyphal systems present in different species. *Polystictus xanthopus* (1932A) was shown to be composed of no less than three series of hyphæ instead of one as was formerly thought to be the case in the polypores. These he named *skeletal*, *binding*, and *generative*, according to their functions. Skeletal hyphæ provided a rigid framework for the hymenophore. Binding hyphæ held the former together and formed the bulk of the tramal walls upon which the hymenium was developed. Generative hyphæ produced the hymenium and surface tissues of the hymenophore.

In a second paper Corner (1932B) showed that in *Fomes levigatus* and *F. senex* there were but two hyphal series—skeletal and generative. He therefore assumed that three hyphal systems were involved in forming fructifications of different species in the family; these were named *monomitic*, *dimitic*, and *trimitic*. The first consisted of generative hyphæ, the second of skeletal and generative, and the third of skeletal, binding, and generative.

While engaged in a study of New Zealand Polyporaceæ and related families an attempt was made by the author to implement delimitation of genera and species by use of those features made known by these brilliant studies of Corner. In a preliminary examination of a score or so of species some rather significant facts emerged which induced a detailed microscopic examination of some two hundred species (of which about one hundred and fifty were polypores). Results, which are summarized herein, show that it is possible to classify species with some exactness on microscopic features alone, and indicate fairly precisely their natural relationships.

### TECHNIQUE

Necessarily some standard method of examination should be followed if others are to secure comparable results. Consequently, the procedure followed during the course of these studies is given below.

Hyphæ of the context were teased apart under a dissecting microscope to ascertain their type, colour, whether septate or aseptate, branched or unbranched, presence or absence of clamp connection, and number of nuclei. Free-hand sections about  $15\mu$  thick were used in studies of the hymenium and related tissues. Sections and teased hyphæ were mounted in a solution of lactic acid (50 per cent. in water), to which had been added 0.1 per cent. analine blue. When heated on the slide over a flame for a few seconds tissues assume an appearance similar to those taken from fresh plants, and mucilage, often present on the hyphæ, is dissolved or rendered transparent so that hyphal details are not obscured. The analine stain colours the contents of spores and basidia without obscuring details of the wall. Skeletal and binding hyphæ seldom stain, but are nevertheless readily seen against the blue background provided by this mountant.

To secure a more precise stain and bring out plasmatic and nuclear details, sections were treated as above, the surplus stain was then removed and a drop or two of aqueous 1 per cent. Safranin O added and heated for a second or two. This was then differentiated for a few seconds with a drop or two of lactic acid-analine blue solution. The surplus was removed with blotting-paper and a cover-slip applied. The double stain gives sharp differentiation of plasmatic contents, and stains nuclei readily. It is fugitive, however, and lasts but a few hours.

It is sometimes not easy to ascertain the specific hyphal system. The following notes may assist in difficult cases: if clamp connections are present in all hyphæ, then the system is monomitic, as is the case if when tissues are teased apart under a dissecting microscope, only one hyphal series or type is seen. The species possesses a trimitic system if clamp connections are present in those with brown-coloured hyphæ of more than one hyphal series. Clamp connections are usually present in generative hyphæ of species with a dimitic system and hyaline hyphæ (save *Fomes*); and invariably absent from those with a dimitic system and brown-coloured hyphæ. Sometimes mucilage is so plentiful in tissues of the context that, despite treatment with lactic acid, hyphæ cannot be examined. It is then advisable to remove the mucilage with a 5 per cent. solution of potassium hydroxide in water. Hyphæ may then be stained with a 1 per cent. aqueous solution of Eosin.

### HYPHAL SYSTEMS

As has been shown by Corner, three well-defined hyphal systems are present in the family. His findings have been amply confirmed in these present studies. Corner (1932A) also referred to *mediate* hyphæ which he at first thought might represent a fourth system. They are merely part of

the skeletal series, however, linking skeletal with generative hyphæ, so need not be further considered. Reichert and Azizar (1939) listed five systems for *Ganoderma lucidum*. Additional to those described by Corner, they added *palisade* and *plectenchyma* systems. Both are involved in formation of palisade tissue which forms the thick, hardened cortex of this species. The condition is confined to relatively few species, so employment of either system has not been considered herein, though doubtless one (palisade) may be useful in further segregation of species with a crustose surface.

*Monomitic*.—Hyphæ are of the generative series alone and "long" type, coloured brown or hyaline, thin-walled, septate, branched, and from  $3\mu$  to  $10\mu$  in diameter. They serve to form the hymenophore in the absence of skeletal or binding series. (Clamp connections may be present or absent.)

*Dimitic*.—Composed of skeletal and generative series, brown or hyaline. (Clamp connections are present (in generative hyphæ) or absent.)

*Trimitic*.—Composed of skeletal, binding, and generative series, brown or hyaline. All species possess clamp connections in the generative hyphæ.

#### HYPHAL SERIES AND TYPES

Hyphæ of the systems may be segregated into definite series and types, which in turn may be used to divide species into related groups.

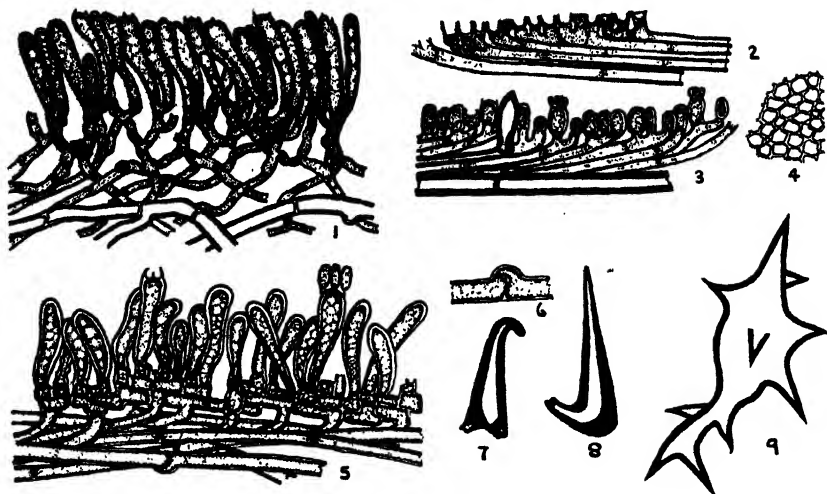
*Generative Series*.—Species with a monomitic system possess hyphæ which are branched, septate, and thin-walled. They may be long and ribbon-like, twisted and collapsed, or, in rare cases, closely interwoven. In diameter they range from  $3\mu$  to  $10\mu$ . Basidia develop into a palisade from branched ends of the tramal hyphæ. Plants with dimitic and trimitic systems possess generative hyphæ, which are, with few exceptions, also freely branched, septate (save in *Fomes annosus*, *F. hemitephrus*, and *Poria subacida*), hyaline (coloured in a few brown species), and stain readily (save for a few species of *Fomes* and *Poria*). They range in diameter from  $1.5\mu$  to  $6\mu$ , most being  $2\mu$ .

*Skeletal Series*.—Two general types of hyphæ are present, herein termed "bovista" and "long."

Bovista types may be dissected out readily, and are common to species with hyaline floccose context, such as *Polyporus betulinus* and *P. sulphureus*. Each consists of a main stem from  $3\mu$  to  $10\mu$  diameter, producing several lateral branches, which in turn may be branched and tapered toward the ends (Fig. 10). They are present in hyaline species with a dimitic system and in both hyaline and brown-coloured species with a trimitic system. The name has been given to hyphæ of this type because of their resemblance to capillitium of the genus *Bovista*.

Hyphæ of the bovista type may be further subdivided into: (a) hyphæ with thin walls and a prominent continuous lumen which usually stains as to contents (septa have been noted in only *Poria dictyopora*, *P. medullaripanis*, and *P. subserpens*); (b) hyphæ with thick walls, the lumen being almost obliterated save for a narrow, sometimes interrupted, thread, spaces at gussets, and occasional cavities in the main stems; even in sections their nature may be ascertained by the varying diameters of hyphal cross-sections (septa have been noted in only *Polyporus braunii*, *P. gramocephalus*, *P. rosettus*, and *Poria vineta*).

The "long" type is composed of slender hyphæ which may ramify in the context for several millimetres, either in a continuous parallel band, or loosely interwoven, when their nature may be ascertained upon dissection (Figs. 1, 3, 5). They range in thickness from  $3\mu$  to  $7\mu$ , and may be equal throughout their length or taper towards the ends. Sometimes the hyphæ become knotted and twisted.



FIGS. 1 TO 9.

Fig. 1.—Section through the meruloid hymenium of *Merulius corium* showing generative hyphae composing the hymenium, and, at the base, two skeletal hyphae.

Fig. 2.—Section through the honeycomb hymenium of *Polyporus tabacinus* with collapsed basidia giving the honeycomb appearance.

Fig. 3.—Section through the hymenium of *Polyporus tabacinus* showing basidia before collapse (two with immature spores). Ventricose seta and skeletal hyphae have thick black walls.

Fig. 4.—Surface view of collapsed basidia of *P. tabacinus* showing the honeycomb appearance.

Fig. 5.—Section through clavate hymenium of *Polyporus betulinus* showing isodiametric cells of the sub-hymenium. Hyphae shown are generative, one having a clamp connection.

Fig. 6.—Clamp connection of *Merulius lacrymans*.

Fig. 7.—Hooked seta of *Peria uncinata*.

Fig. 8.—Ventricose seta of *Polyporus tabacinus*.

Fig. 9.—Compound seta of *Polyporus gramineocephalus*.

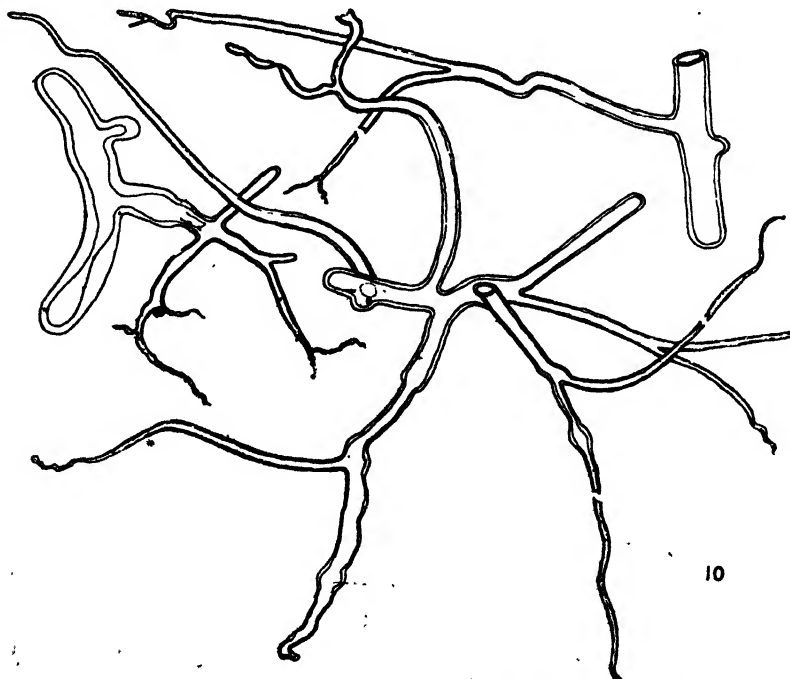


FIG. 10.—Bovista type of skeletal hyphae of *Polyporus eucalyptorum*. Three specimens have been drawn to show diversity of the hyphae present in one plant.

They may be further differentiated according to whether they are septate or aseptate, branched or unbranched, or possess thick or thin walls. Those with thick walls possess an almost obliterated lumen. The type is common to both hyaline and brown-coloured species with dimitic and trimitic systems.

*Binding Series.* Present in species with a trimitic system, binding hyphæ like those of skeletal—consist of the two general types, "bovista" and "long." Hyphæ of the "bovista" type are freely branched, aseptate, and range in diameter in the main stem from  $3\mu$  to  $9\mu$ . "Long" types may be sparingly or freely branched, seldom tapered at the ends, and in diameter range from  $2\mu$  to  $4\mu$  most being about  $4\mu$ .

#### CLAMP CONNECTIONS

Present in all hyaline-coloured species with a monomitic system (Figs. 5, 6), clamp connections are absent from those with brown hyphæ, save *Merulius lacrymans*. They are also present in most hyaline species with a dimitic system, save *Fomes annosus*, *F. hemitephrus*, and *Poria subacida*, the last being a resupinate *Fomes*. Clamps have not been seen in one group of hyaline-coloured species with a dimitic system, since the hyphæ form a convoluted, almost sclerotoid, context which prevents threads being dissected out for examination. Species of the group are *Polyporus braunii*, *P. catervatus*, *P. colensoi*, *P. infernalis*, and *P. proprius*. In all probability they are present since they have been noted in one species with similar context, *Polyporus rosettus*.

Clamp connections are absent from brown-coloured species with a dimitic system, which suggests there is a fundamental difference between species with brown and those with hyaline hyphæ. On the other hand, they are present in all species examined with a trimitic system whether possessing brown or hyaline hyphæ. This, again, suggests that the hyphal systems are significant.

These bodies are often difficult to detect, and sometimes they are limited to the sub-hymenial layer of young plants (Fig. 1). In other cases they may be seen readily, especially in species with a monomitic system possessing hyaline-coloured hyphæ, and in the brown hyphæ of *Merulius lacrymans*. As clamp connections are associated with nuclear behaviour, efforts were made to ascertain the number of nuclei in each hyphal cell. Nuclei were seen in only a few species. Most are extremely small, being about  $0.2\mu$  diameter, and consequently are easily overlooked. Species in which nuclei were seen fall into the following groups:—

##### *Binucleate*—

Monomitic, hyphæ hyaline, clamp connections present—

*Polyporus adustus*, *P. fumosus*.

Dimitic, hyphæ hyaline, clamp connections present—

*Merulius confusum*, *M. corium*, *M. miniatus*.

*Polyporus rosettus*.

*Poria selecta*, *P. sinuosa*, *P. vaporaria*, *P. vincla*.

Trimitic, hyphæ hyaline, clamp connections present—

*Trametes cinnabarina*.

*Polyporus versicolor*.

##### *Uninucleate*—

Dimitic, hyphæ brown, clamp connections absent—

*Poria contigua*, *P. ferruginosa*, *P. luteo-fulva*.



While it is perhaps unwise to generalize on such a few records, it is a reasonable assumption that clamp connections are connected with the number of nuclei, being present in binucleate hyphæ and absent from uninucleate.

#### HYPHAL COLOUR

Colour of the context has been used by several workers as a generic character. There is some justification for separating those with brown from those with hyaline hyphæ; for, as has been shown, hyphal colour appears to be associated with presence or absence of clamp connections. There is little to support use of bright colours or of different shades of brown in generic delimitation. *Trametes cinnabarina*, for example, is described as having cinnabar-red context, and on this score was placed under *Pycnoporus* by Murrill. Under the microscope hyphæ are seen to be hyaline, with bands of reddish- or orange-coloured pigment granules deposited at intervals on portions of the hyphal walls. The species therefore possesses hyaline hyphæ and should be grouped accordingly. Many species with yellow or light-brown context owe their colour to deposits of brown or yellow mucilage coating the exterior of the hyphæ, or embedding them. When deposits are removed with lactic acid or potassium hydroxide, hyphæ are seen to be hyaline. Again, it is a common experience to find species with white context when fresh change in colour to isabelline or even ochraceous brown when dry. Lloyd (1915) created several sections to contain species which had undergone such a change, with the result that closely related plants were sometimes placed in different sections. Others with context coloured pink or rose are seen under the microscope to possess hyphæ which are either hyaline or light brown.

Because of these discrepancies, the author has described hyphal colour as seen under the microscope when tissues were viewed with apochromatic objectives and monochromatic light. All then fall into two groups—those with hyphæ coloured some shade of brown, and those which are definitely hyaline as to the hyphal wall. Difficulty was experienced in placing very few. *Poria attenuata*, *Lenzites betulina*, *Fomes ellisianus*, and *F. roseus* possess context hyphæ which are tinted brown, so might be included with hyaline-coloured species unless critically examined under the conditions specified.

#### BASIDIAL TYPES

Three general types of basidia and related structures are present in species of the family. Herein they have been named “merulioid,” “honeycomb,” and “clavate.” Basidia arise from a sub-hymenium which may be composed of one or more layers of hyphæ lying parallel with tramal walls, of densely woven hyphæ partly gelatinized, or of two or more layers of isodiametric cells. They may persist through the life of the plant, or collapse as soon as spores are shed.

*Merulioid Type*.—Basidia are hyaline, long-cylindrical, from  $12\mu$  to  $30\mu$  long,  $3\mu$  to  $4\mu$  broad, persistent (Fig. 1), and compacted into a gelatinous firm layer usually arising from a sub-hymenium of densely woven hyphæ sometimes partly gelatinized when it is then difficult to identify individual hyphæ. Spores are usually cylindrical, or allantoid, exceptions occurring in a few species of *Merulius*. Basidia and the sub-hymenial tissue may usually be separated from the tissues of the context. This feature was used by Montagne as the generic character of *Gloeoporus*. It is also common to species of *Merulius*, hence the name for the basidial type.

*Honeycomb Type.*—Basidia are clavate or oval, short, usually about  $8\mu \times 4\mu$ , hyaline, stain readily, and are laterally firmly cemented at the base into a palisade (Fig. 3). Finally they collapse, leaving the cemented basal layer persistent on the tramal wall, where it has an appearance not unlike the surface of honeycomb (Fig. 2), and occasionally shows the edges, where cut with the razor, perforated like an edge of a postage-stamp (Fig. 4). Of the 32 species exhibiting this basidial type, all but three possess brown-coloured hyphæ, exceptions being species of *Fomes* with hyaline context.

*Clavate Type.*—Most species possess this type. Basidia are usually clavate, less commonly elongate-clavate or fusoid, of varying lengths,  $6\mu$  to  $25\mu$ , and arise from a two or three layered sub-hymenium (Fig. 5). They may be compacted into a dense palisade, arranged loosely and irregularly upon the sub-hymenium or, following spore discharge, collapse and become gelatinized, then appearing in old plants as a formless mucilaginous film overlying the tramal wall. Basidia are not infrequently mixed with ends of skeletal hyphæ, which sometimes project beyond the hymenial layer into the pore cavity and have then been termed paraphyses or cystidia.

#### INCLUSIONS

*Setæ.*—Many species with brown-coloured hyphæ possess ventricose, thick-walled, chestnut-brown setæ embedded in the hymenium or context (Figs. 7 and 8). They arise from skeletal hyphæ in species with a dimittic system, from generative in species with a monomittic system, such as *Polyporus cuticularis* and *P. radiatus*. Setæ have not been seen in plants with a trimitic system. Usually setæ develop below the sub-hymenium and protrude beyond the basidia. In size they range from  $10\mu$  to  $160\mu$  in length, and in diameter from  $6\mu$  to  $12\mu$ . Bodies of similar colour but much greater length may be seen in the context of a few species, an example being *Polyporus tabacinus*. In *Polyporus gramocephalus* they are compound, and form conspicuous objects against the hyaline context (Fig. 9).

*Cystidia.*—Few species of the family possess these bodies, so characteristic a feature in certain of the Thelephoraceæ. Gloecocystidia have been noted in *Polyporus braunii*, *Merulius miniatus*, *Poria attenuata*, and *P. vineta*. Usually hyaline smooth hyphæ with coloured lumen, they are encrusted in *Polyporus braunii* and *Poria vineta*, appearing in section not unlike a maize-cob.

*Lactiferous Ducts.*—Present in *Fomes annosus*, *Polyporus berkeleyi*, *P. colensoi*, *P. lateritius*, *P. proprius*, and *P. sulphureus* of the species examined, ducts are not easily seen unless sections are stained. They then become conspicuous objects, extending in the context for a length of  $200\mu$  or more. Some may be convoluted, others resemble spirochaetes or giant hyphæ, or may even appear stellate.

*Crystals.*—A few species exhibit abundant crystals embedded between the hyphæ of the context. Their presence usually renders sectioning difficult. Crystals may be small (*circa*,  $10\mu$ ) and globose or cubical, or irregular masses attaining a diameter of  $50\mu$  or more. In species of *Merulius* they form a definite layer beneath the hymenium, making its separation from the context relatively easy. Crystals have also been noted in *Trametes abietis*, *Fomes roseus*, *Polyporus berkeleyi*, *P. calervatus*, *P. poculus*, *P. proprius*, *Poria calcea*, *P. hyalina*, *P. subacida*, and *P. tarda*.

*Mucilage.*—The context is stiffened into a caseous consistency in a few species by mucilaginous or gelatinous matter in which the context hyphæ are embedded. *Polyporus australiensis*, *Fomes laricis*, *Poria macrospora*, and *P. xantha* are typical examples.

Save for the ventricose setæ present in a few species with brown-coloured hyphæ it is doubtful if inclusions are of classifiable significance, because of their rarity. When present, as the maize-cob cystidia of *Polyporus braunii* and *Poria vincla*, they serve as useful aids in specific diagnosis.

By use of the characters discussed it has been possible to arrange the species studied into numerous natural groups which indicate their relationships. These features afford a more exact means of separation than is possible by use of the macroscopic characters used by Fries, since form and texture depend upon the hyphal systems and hyphal types present in all species. Corner (1932B, p. 72) has ably summarized the position thus (*italics being mine*):—

. . . . The fruit bodies . . . . differ . . . . as one would expect, in the properties of the hyphal wall, in the modification of the hyphal ends on cessation of growth, in the behaviour and manner of branching of the hyphæ and in the factors governing the relative development of the tissues. These are seldom recorded in specific descriptions: reliance is had rather on macroscopic characters, *which are but secondary effects of the minute construction with little or no mycological significance*. Differences in colour and texture are determined by the characters of the crust and the hyphal systems; the microscopic structure of the upper surface decides whether it will be smooth, matt, velutinate, tomentose, laccate, and so forth. Such descriptive terms would serve if they really indicated definite microscopic characters, but in referring only to the gross appearance they are too vague. A matt surface, for instance, may be caused by the crowding of the ends of the skeletal hyphæ at a common level, by the development of an irregular crust by the generative hyphæ, or merely by the excretion of granular and crystalline matter: a woody or corky texture may be due to the abundance of oblique and transverse skeletal hyphæ, or to the presence of a binding system as well, or even to a single system of hyphæ with thickened walls and compact arrangement. And should such fundamental differences reach a macroscopic level they can be expressed only very inadequately in degrees of woodiness, heaviness, roughness, or hardness most difficult to appreciate. *Hence it is almost impossible to identify a polypore with certainty from the customary descriptions*: a thorough microscopic examination will often disclose constant peculiar details in otherwise similar fungi that the type material must be re-examined to decide the issue . . . . The Polyporaceæ are acknowledged among the most perplexing fungi to identify. The reason is surely because their detailed study has scarcely begun: only by carrying the microscope to all points can one try out the limitations of a morphological classification.

In subsequent papers, covering taxonomy of New Zealand species of this and related families, use will be made of the microscopic features discussed above to outline a classification which will more closely indicate relationships of species and their generic limitations than has hitherto been possible by use of macroscopic features. Unfortunately, it has not been possible—owing to lack of sufficient material—to examine all traditional species of Europe and North America which have been used as types for the many genera proposed.

The following outline covers some one hundred species (many similar ones being omitted to save space), and should serve as some indication as to the manner in which species fall into natural groups. Many of the traditional genera of Fries will necessarily have to be subdivided into several, since it is evident that under *Polyporus*, *Fomes*, or *Poria* one cannot maintain species with such diverse hyphal systems as monomitic, dimitic, and trimitic; hyaline or brown hyphæ; or types of basidia such as have been outlined. Examinations have been made of specimens contributed by mycologists from Sweden, Germany, France, Italy, England, United States of America, Malaya, Philippines, and Australia, together with those collected over the past quarter-century in New Zealand. Names used are those under which collections were received, and in some cases may be synonymous with others mentioned.

## OUTLINE OF MICROSCOPIC FEATURES IN SPECIES EXAMINED

MONOMITIC.—Generative hyphæ alone present in the context; long, branched, septate, thin-walled:—

Context hyphæ hyaline under the microscope; clamp connections present—

Basidial type merulioid—

*Polyporus dichrous*, *P. phlebophorus*.

*Poria merulina*.

Basidial type honeycomb: no representative seen.

Basidial type clavate—

*Polyporus adustus*, *P. anthracophilus*, *P. atrostrigosus*, *P. fumosus*.

*P. spumens*.

Context hyphæ brown under the microscope—

Basidial type merulioid; clamp connections present—

*Merulius lacrymans*.

Basidial type honeycomb; clamp connections wanting; ventricose setæ present in the hymenium—

*Polyporus cuticularis*, *P. radiatus*.

Basidial type clavate; clamp connections wanting; ventricose setæ wanting—

*Polyporus aureofulvus*, *P. oblectans*, *P. perennis*.

DIMITIC.—Skeletal and generative hyphæ present in the context:—

Context hyphæ hyaline; ventricose setæ absent—

Basidial type merulioid; clamp connections present—

Skeletal hyphæ of bovista type, thick-walled, aseptate—

*Poria selecta*.

Skeletal hyphæ of long type, branched, septate—

*Polyporus pendulus*.

\**Merulius confusus*, *M. corium*, *M. miniatus*.

*Poria hyalina*.

†*Laschia intestinalis* (skeletal hyphæ aseptate).

Basidial type honeycomb; clamp connections wanting; skeletal hyphæ long, branched—

*Fomes annosus* (skeletal hyphæ septate, generative hyphæ aseptate).

*Fomes hemitephrus* (skeletal and generative hyphæ aseptate).

Basidial type clavate; clamp connections present—

Skeletal hyphæ of bovista type—

Skeletal hyphæ septate—

Skeletal hyphæ thin-walled—

*Poria dictyopora*, *P. medulla-panis*, *P. subserpens*.

Skeletal hyphæ thick-walled, lumen almost obliterated—

†*Polyporus braunii*, *P. grammacephalus*, *P. rosettus*.

*Poria vineta*.

Skeletal hyphæ aseptate—

Skeletal hyphæ thin-walled—

*Polyporus betulinus*, *P. eucalyptorum*, *P. sulphureus*.

Skeletal hyphæ thick-walled, lumen almost obliterated—

*Polyporus arcularius*, *P. berkeleyi*, †*P. infernalis*, *P. volvatus*,

*P. xerophyllus*.

*Poria cinerescens*.

*Trameles hexagonoides*.

Skeletal hyphæ of long type—

Skeletal hyphæ septate—

Skeletal hyphæ branched—

*Polyporus australiensis*, †*P. catervatus*, *P. varius*.

††*Poria subacida*.

Skeletal hyphæ unbranched—

*Fomes officinalis*.

Skeletal hyphæ aseptate—

Skeletal hyphæ branched—

*Polyporus benzoinus*, †*P. colensoi*, †*P. proprius*.

*Poria calcea*.

Skeletal hyphæ unbranched—

*Polyporus alutaceus*, *P. borealis*.

*Poria sinuosa*, *P. vaporaria*, *P. xantha*.

\* Manuscript name for species not yet identified.  
† *Fomes* type with pores in strata.

† Clamp connections not seen since

context is sclerotoid.

DIMITIC.—Skeletal and generative hyphæ present in the context—*continued*

Context hyphæ brown; clamp connections wanting—

Basidial type meruloid: no representative seen.

Basidial type honeycomb; skeletal hyphæ of long type—

Ventricose setæ present in the hymenium—

Skeletal hyphæ septate—

Skeletal hyphæ branched—

*Fomes putearius*, *F. rimosus*.‡*Poria ferruginosa*, ‡*P. subweirii*.*Trametes pini*.

Skeletal hyphæ unbranched—

*Fomes ignarius*, *F. pomaceus*, *F. robustus*.*Polyporus gilvus*, *P. tabacinus*.

Skeletal hyphæ aseptate—

Skeletal hyphæ branched—

*Poria brunneo-adhaerens*, ‡*Poria ferrea*.‡*Poria setosa*, *P. subcontigua*, ‡*P. uncinata*.

Skeletal hyphæ unbranched—

*Fomes senex*, *F. zeylandicus*.*Trametes isabellinus*.‡*Poria pereffusa*, ‡*P. punctata*.

Ventricose setæ wanting—

Skeletal hyphæ septate—

Skeletal hyphæ branched—

‡*Poria carteri*.

Skeletal hyphæ unbranched—

*Polyporus* “*lateritius*.”

Skeletal hyphæ aseptate—

Skeletal hyphæ branched—

*Fomes applanatus*.

Skeletal hyphæ unbranched—

‡*Poria friesiana*.

Basidial type clavate; skeletal hyphæ of long type—

Ventricose setæ present in the hymenium—

Skeletal hyphæ branched, septate—

‡*Trametes abietis*.

Skeletal hyphæ branched, aseptate—

*Poria contigua*.

Ventricose setæ wanting—

Skeletal hyphæ branched, septate—

*Polyporus ludovicianus*.*Hexagonia gunnii*.*Poria luteo-fulva*.

Skeletal hyphæ branched, aseptate—

\**Fomes awahitu*, *F. ellisianus*, *F. marmoratus*.‡*Poria attenuata*.

Skeletal hyphæ unbranched, aseptate—

*Poria carbonaria*.

## TRIMITIC.—Skeletal, binding, and generative hyphæ present in the context; clamp connections present; ventricose setæ wanting—

Context hyphæ hyaline—

Basidial type meruloid: no representative seen.

Basidial type honeycomb. Skeletal hyphæ long, aseptate, unbranched; binding hyphæ of bovista type—

*Fomes lignosus*.

Basidial type clavate. Skeletal hyphæ aseptate, unbranched, long—

Binding hyphæ of bovista type, aseptate—

*Polyporus affinis*, *P. hirsutus*, *P. occidentalis*, *P. versicolor*, *P. zonatus*.*Trametes cimabarina*, *T. persoonii*, *T. suavolens*.

Binding hyphæ of long type, aseptate, sparingly branched—

*Fomes fraxinophilus*.*Daedalea confragosa*, *D. unicolor*.*Poria macrospora* (binding hyphæ unbranched).

\* Manuscript name for species not yet identified.

‡ *Fomes* type with pores in strata.

TRIMITIC.—Skeletal, binding, and generative hyphæ present in the context; clamp connections present: ventricose setæ wanting—*continued*

Context hyphæ brown. Skeletal hyphæ long, aseptate, unbranched—

Basidial type merulioid: no representative seen.

Basidial type honeycomb. Binding hyphæ of long type, aseptate, branched—

*Fomes lucidus*, *F. pinicola*, *F. roseus*.

Basidial type clavate—

Binding hyphæ of bovista type, branched, aseptate—

*Lenzites adustus*, *L. betulina*, *L. corrugata*, *L. saepiaria*.

*Fomes fomentarius*.

*Trameles hispida*, *T. mollis*.

Binding hyphæ of long type, aseptate—

*Fomes marginatus*, *F. palliseri*.

\**Daedalea ovaivaka*, *D. quercina*.

†*Trameles odorata*.

\* Manuscript name for species not yet identified

† *Fomes* type with pores in strata

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## COPPER COMPOUNDS AS FERTILIZERS FOR PASTURES DEFICIENT IN COPPER

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### Summary

It has been shown that commercial bluestone applied as a fertilizer increases the copper content of deficient pastures grown on peat soils and prevents stock-diseases due to copper deficiency. The most suitable rate of application is 5 lb. per acre each year, and autumn top-dressing is more effective than spring top-dressing.

A single top-dressing causes a marked response in the first year, but after twelve months the pasture copper has dropped to a level slightly above that of untreated pastures and remains at that level for at least two more years. There is a higher maximal and more persistent effect on peat soils than on clay soils.

Repeated annual top-dressings even at as high a rate as 20 lb. per acre do not cause abnormally high pasture copper contents.

Insoluble copper oxychloride is no more effective as a fertilizer than soluble copper sulphate.

The copper content of hay is increased by top-dressing in the spring after the hay paddock has been shut up.

Copper sulphate does not damage the pasture crop; there is, in fact, a tendency for copper fertilizers to favour the persistence and establishment of the better types of pasture species on copper-deficient land.

### INTRODUCTION

THE most effective and reliable method of supplying copper to stock on the copper-deficient peat areas of New Zealand has been to top-dress the pastures with copper sulphate or with fertilizers containing copper. The method ensures that all grazing stock obtain copper regularly, and has the additional advantage that the nutritional environment of the pasture is brought nearer to normal.

Several important points required study before top-dressing with copper compounds could be employed with confidence and on a wide scale over copper-deficient areas. It was necessary, for example, to determine (a) the minimum effective rate of top-dressing and the optimum intervals between top-dressings and the maximum safe rate of top-dressing, (b) if repeated top-dressings of the same area result in dangerous accumulation of copper, (c) if other fertilizers applied at the same time influence the uptake of copper by the pasture plant, (d) if time of year in which top-dressing is applied affects the result, and (e) if there is any effect on the botanical composition of the pasture cover.

Practically no assistance on these points could be obtained from the literature. It is true that copper has been employed to a considerable extent in America, Australia, and Europe as a fertilizer for crops such as cereals, tomatoes, potatoes, peas, and to control exanthema and die-back in citrus and other fruit trees. As a top-dressing for pastures, however, very limited

use indeed has been made of copper compounds. In the Gingin area of Western Australia, Bennetts and Beck(1) showed that 20 lb. copper sulphate per acre increased to normal the copper content of deficient pastures and that the effect persisted for at least one and a half years, and Underwood, Robinson, and Curnow(2) found that 15 and 7½ lb. copper sulphate were effective for three years and two years respectively. The soil in Gingin is a sand type, whereas, in New Zealand, copper-deficient areas are peat soils in various stages of maturity.

It was obviously necessary to institute a comprehensive plan of experimental work which would give the necessary information and give it under the existing local conditions. This paper reports the results that have been obtained for the first three years of the investigation.

### EXPERIMENTAL

In each of two typical peat areas a series of small plots was laid out. The plots were top-dressed annually with copper compounds, alone or in combination with other fertilizers. Copper sulphate in the form of commercial bluestone was used for most of the plots, because the advantages of cheapness and availability commended it for later large-scale use in commercial farming. In one season certain plots were top-dressed with Cuprox, a commercial preparation containing 97 per cent. copper oxychloride, which differs from copper sulphate in being insoluble in water. The bluestone employed contained 24·8 per cent. of copper and the Cuprox 50·4 per cent. of copper.

A small number of plots at Wallaceville were also top-dressed to give some data on the effect of application of copper to pasture on soil containing no peat.

Pasture samples were taken at approximately monthly intervals from all the plots and analysed for copper.

Regular samplings were also made of pastures on fields varying in size from 6 to 50 acres to which bluestone or Cuprox had been applied in the course of experiments on the control of stock diseases caused by copper deficiency.

Details of the experimental procedure and of the results are as follows :—

#### *Plot Experiments*

Plots on peat land were situated at Waitakaruru and on an area in the Gordonton district, near Hamilton. The Waitakaruru area was of moderately consolidated peat on which the pasture was almost entirely Yorkshire fog (*Holcus lanatus*) and *Lotus major*. Gross copper deficiency existed, the mean copper content of a large number of pasture samples taken at different seasons of the year being 3·6 p.p.m. Cattle and sheep livers were extremely low in copper. The cattle were unthrifty, and enzootic ataxia occurred in lambs. The Gordonton area was of relatively well-consolidated peat soil and the pasture was predominantly Yorkshire fog, with perennial rye-grass and white clover constituting about 40 per cent. of the cover. Severe peat scours occurred in the dairy stock and the copper content of the livers was low. The mean copper content of a large number of pastures taken at different seasons of the year was 7·1 p.p.m.

The mean copper content of "normal" New Zealand pastures has been found to be 11·0 p.p.m.



The layout of the plots is shown in Fig. 1.

—			B.	L.	S.	P.	B.	L.	S.	P.	B.	L.	S.	B.	L.	
			(1)				(2)				(3)				(4)	
1943	..	..	0	20	3	1	..	..	..	..	0	0	3	0	..	
1944	..	..	2½	0	3	1	..	..	..	..	2½	2	..	2½	2	
1945	..	..	2½	0	3	1	..	..	..	..	2½	2	..	2½	2	
			(5)				(6)				(7)				(8)	
1943	..	..	5	20	3	1	5	..	3	..	5	..	3	5	..	
1944	..	..	5	..	3	1	..	..	..	..	5	2	..	5	2	
1945	..	..	5	..	3	1	..	..	..	..	5	2	..	5	2	
			(9)				(10)				(11)				(12)	
1943	..	..	10	20	3	1	10	..	3	..	10	..	3	10	..	
1944	..	..	10	..	3	1	..	..	..	..	10	2	..	10	2	
1945	..	..	10	..	3	1	..	..	..	..	10	2	..	10	2	
			(13)				(14)				(15)				(16)	
1943	..	..	20	20	3	1	20	..	3	..	20	..	3	20	..	
1944	..	..	20	..	3	1	..	..	..	..	20	2	..	20	2	
1945	..	..	20	..	3	1	..	..	..	..	20	2	..	20	2	
			(17)				(18)				(19)				(20)	
1943	..	..	..	..	..	..	..	..	..	..	..	..	..	40	..	
1944	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
1945	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
			(21)				(22)				(23)				(24)	
1943	..	..	..	..	..	..	..	..	..	..	..	..	..	5*	..	
1944	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
1945	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
			(25)				(26)				(27)				(28)	
1943	..	..	..	..	..	..	..	..	..	..	..	..	..	10*	..	
1944	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
1945	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
			(29)				(30)				(31)				(32)	
1943	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
1944	..	..	2½	2	..	..	5	2	..	..	10	2	..	20	2	
1945	..	..	2½	2	..	..	5	2	..	..	10	2	..	20	2	

\* Indicates Cuprox.

FIG. 1.—Plan of Experimental Plots at Waitakaruru and Gordonton, showing Top-dressing applied in Different Years.

(B = bluestone (pound per acre) ; L = agricultural lime (hundredweight per acre) ; S = serpentine-superphosphate (hundredweight per acre) . P = muriate of potash (hundredweight per acre).)

Two sets of plots, each the same as that illustrated in the figure, were employed in each area. Duplicate observations were therefore obtained at Waitakaruru and at Gordonton. The plots were totally enclosed by a stock-proof fence approximately 15 ft. outside the plots. The belt between the plots and the fence was not top-dressed and provided untreated pasture samples for comparison with pastures from top-dressed areas. The dimensions of each plot were 20 × 25 links and the area  $\frac{1}{10}$  acre.

Up to the present time the plots have been under observation for three years, 1943 to 1945. The top-dressing applied each year is shown on each plot in Fig. 1.

All 1943 top-dressing was done in the autumn—at Waitakaruru on 14th April, 1943, and at Gordonton on 2nd March, 1943. In 1944 and 1945 all plots except 3, 7, 11, and 15 were top-dressed in the autumn—at Waitakaruru on 18th February, 1944, and 22nd March, 1945, and at Gordonton on 15th February, 1944, and 23rd March, 1945. Plots 3, 7, 11, and 15 were top-dressed in the spring in 1944 and 1945—at Waitakaruru on 20th September, 1944, and 17th September, 1945, and at Gordonton on 18th September, 1944, and 10th September, 1945.

The layout of the plots permitted the following observations to be made :—

- (a) A comparison of plots 1, 5, 9, and 13 with plots 4, 8, 12, and 16 showed the differences between the uptake of copper when bluestone, mixed with serpentine-superphosphate and potash, was applied at different rates to heavily limed land and when it was applied with small quantities of lime alone.
- (b) A comparison of plots 3, 7, 11, and 15 with plots 4, 8, 12, and 16 showed the differences between the effect of spring and autumn top-dressing with copper at different rates of top-dressing.
- (c) Continued observations on plots 6, 10, 14, 20, and 28 showed the persistence of effect of one top-dressing of different amounts of bluestone or Cuprox on copper content of pasture.

Observations on the foregoing were incomplete for the first year after top-dressing, but are complete for the two years after the second and third top-dressings.

Complete data from the first top-dressing is presented for plots 29, 30, 31, and 32. These plots were not top-dressed in 1943, but were top-dressed in 1944 and 1945 at the rates of  $2\frac{1}{2}$ , 5, 10, and 20 lb. bluestone per acre respectively.

The plots at Wallaceville were on a clay soil with a pasture cover of rye-grass and white clover. The area of each plot was  $\frac{1}{35}$  acre.

Top-dressing was carried out in the autumn of 1944 and 1945 on 14th April, 1944, and 3rd April, 1945. Each plot received lime at the rate of 2 cwt. per acre, and in a group of five plots the rates of application of bluestone were 0,  $2\frac{1}{2}$ , 5, 10, and 20 lb. per acre. There were two such groups in the experiment.

All plots were sampled at approximately monthly intervals and analysed for copper by methods already described(3). After each sampling the plots were rapidly grazed off by stock drawn from pastures not top-dressed with copper. The droppings were spread by light harrowing. Grazing was so managed that pasture was 4 in. to 6 in. in length when the date for the next sampling came round. Usually one grazing between sampling dates was sufficient, but more were necessary in seasons of rapid pasture growth.

Results are shown in graphical form in Figs. 2 to 4. The copper contents of the pastures are expressed as parts per million copper in dried grass, and in preparing the graphs the mean has been employed of two equivalent duplicate plots in the one location for each date.

#### *Samples from Top-dressed Fields*

On four farms top-dressing was carried out as a prophylactic measure against enzootic ataxia and peat scours.

One of the farms was the peat land adjacent to the Waitakaruru plots.

On this farm one field was top-dressed with a single application of 20 lb. Cuprox and 2 cwt. lime per acre, a second field was top-dressed with 5 lb. bluestone in 2 cwt. lime the first year and 5 lb. bluestone in 2 cwt. superphosphate the second year, and a third field was top-dressed in two successive years with 10 lb. bluestone and 2 cwt. lime per acre. Results are shown in Fig. 5A.

The second farm was that on which the Gordonton plots were located, and the remaining two were similar in history to this farm and were situated in Ngatea and Pongakawa. On these farms the whole area was top-dressed and normal farming methods were followed. Five pounds of bluestone and 2 cwt. lime per acre were applied to most fields, 10 lb. bluestone and 2 cwt. lime were applied to one field on each of two farms, and 20 lb. bluestone and 2 cwt. lime were applied to one field on each of the three farms.

The mean result for each different rate of top-dressing is shown in Fig. 5B.

### Results

*Waitakaruru Plots.*—Figure 2A: This depicts the results for plots 29, 30, 31, and 32 in comparison with the untreated control area, and shows the effects of autumn top-dressing in two successive years with bluestone at the rates of  $2\frac{1}{2}$ , 5, 10, and 20 lb. per acre applied with carbonate of lime at 2 cwt. per acre. As little as  $2\frac{1}{2}$  lb. bluestone per acre produced an appreciable effect, the copper content of treated pastures being more than twice that of the controls. With increased weight of bluestone applied, up to 10 lb. per acre, there was increased uptake of copper by the pasture, but this effect was not directly proportional to the greater weight of copper in the heavier dressings. The difference between the effects of 10 and 20 lb. per acre was insignificant.

Figure 2B: This gives a comparison of the results for plots 5 and 8 and for 13 and 16 and also shows results for pasture from the untreated control area. The purpose of the comparison was to determine the effect of applying bluestone in conjunction with different fertilizers. Of the pairs enumerated above, the first named (continuous lines in graph) in each instance was top-dressed in 1943 with carbonate of lime at the rate of 1 ton per acre and bluestone was applied mixed with serpentine-superphosphate and muriate of potash in 1943, 1944, and 1945. The second plot of each pair (dotted lines in graph) was top-dressed in 1943 with bluestone alone and in 1944 and 1945 with bluestone mixed with 2 cwt. carbonate of lime per acre. The two pairs shown in Fig. 2B are for dressings of bluestone of 5 and 20 lb. per acre. To preserve clarity of presentation, the results for plots 9 and 12, top-dressed with 10 lb. bluestone per acre, have not been included in the graph. The curves for results from these plots fall between those for the 5 and 20 lb. bluestone dressings.

The graph is incomplete for results after the first top-dressing, but is complete for the second and third years' top-dressing.

It will be observed that the copper contents of pastures from pairs of plots are closely similar. Absorption of copper by the plant was the same, whether bluestone was applied mixed with serpentine-superphosphate and potash or with lime.

The graphs also give information on the effect of successive annual top-dressings on the same area. The pastures on all four plots contained less than 10 p.p.m. of copper in the dry grass in November, 1945, at which time there had been three successive annual top-dressings with 5 lb. or 20 lb. of bluestone per acre. At this stage, therefore, there is no evidence of dangerous accumulation of copper in the herbage.



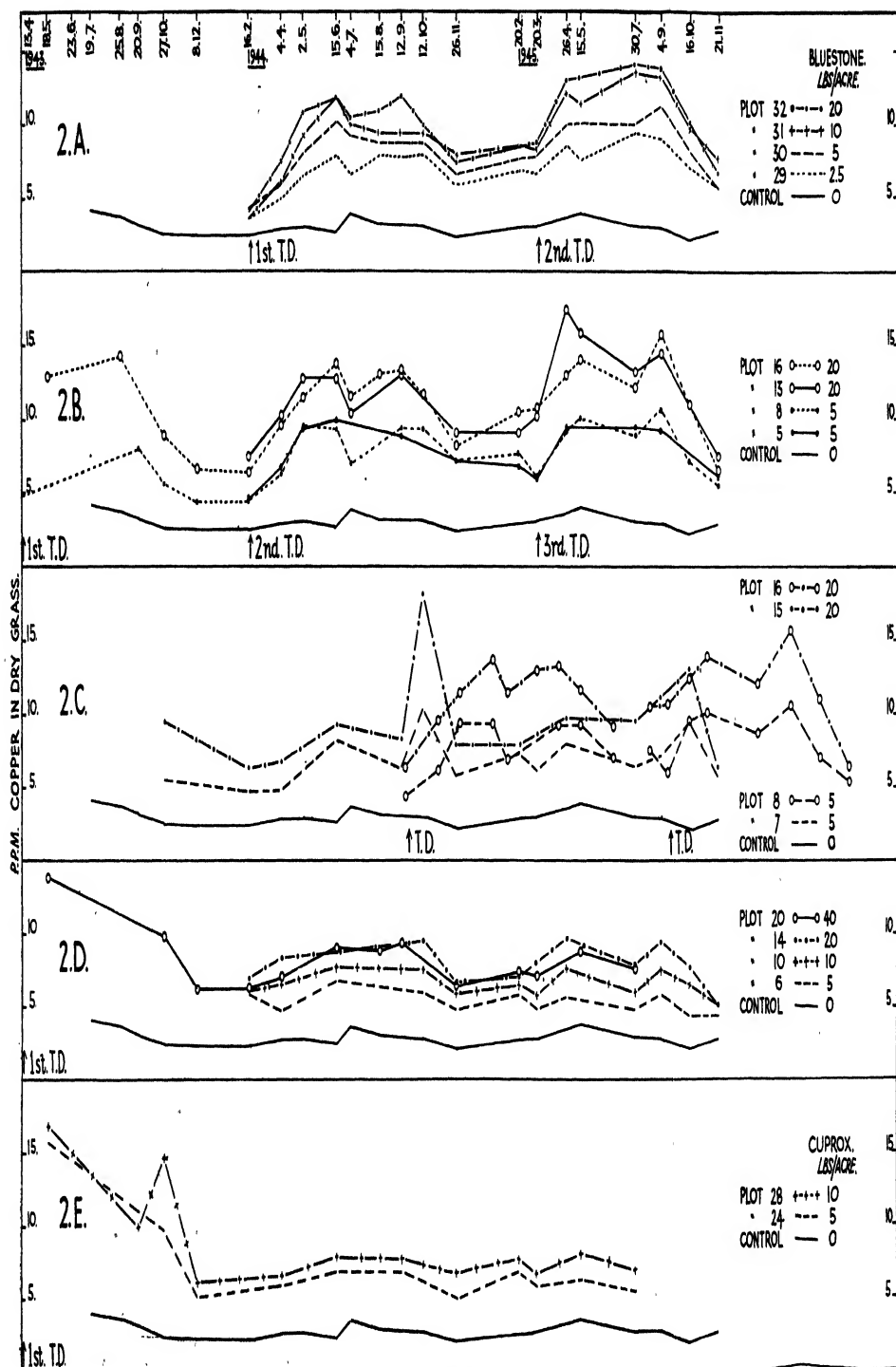


FIG. 2.—Waitakaruru plots.

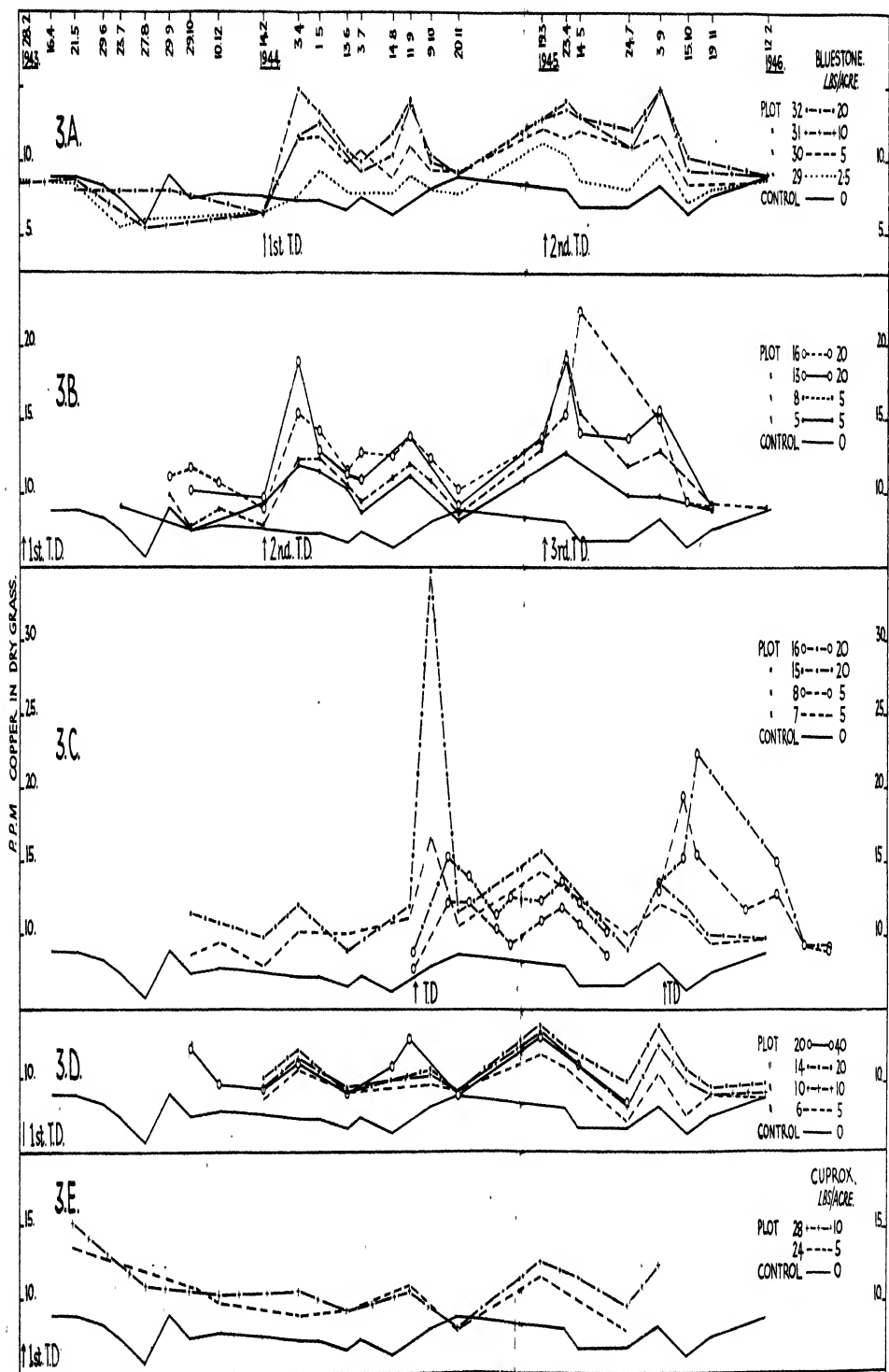




Figure 2c: This graph gives a comparison of the effects of spring and autumn top-dressing. Data for plots 7 and 8 (5 lb. bluestone per acre) and 15 and 16 (20 lb. bluestone per acre) are shown. The method of presenting the results is to show the figures for spring top-dressed plots (7 and 15) as a continuous graph in each case. The results for the corresponding autumn top-dressed plots (8 and 16 respectively) are transposed so that the top-dressing dates for both spring and autumn are made to fall on the same ordinate. Graphs for spring and autumn top-dressed plots for the same rate of bluestone application have the same symbol, except that large circles appear at intervals in the graph for the autumn top-dressed plots.

The results indicate that a more persistent effect is achieved by top-dressing in the autumn. In 1944 there was a sharp rise in pasture copper after spring top-dressing, followed by a rapid fall. The autumn top-dressing produced a slower rise and the increased copper contents were more sustained. In 1945, spring top-dressing did not produce such a high peak in copper content and the fall in copper content was more rapid than after autumn top-dressing in the same year.

Figures 2D and 2E: These graphs show the persistence of the effect of one application of bluestone or Cuprox, the effect being observed for three years after top-dressing on 14th April, 1943. The results for plots top-dressed with bluestone at 5, 10, 20, and 40 lb. per acre are shown in Fig. 2D and for 5 lb. and 10 lb. Cuprox per acre in Fig. 2E.

The effect of as little as 5 lb. per acre was still apparent three years after top-dressing, when the copper content of the pasture was approximately 5 p.p.m., as against a content of 3 p.p.m. in untreated pasture.

One top-dressing with 10, 20, or 40 lb. bluestone per acre produced levels of copper in the pasture persistently higher than that caused by 5 lb. per acre.

On the particular type of soil at Waitakaruru one top-dressing at 20 lb. bluestone per acre would maintain the copper content of the pasture for at least three years at a sufficiently high level to ensure stock health.

One top-dressing with 5 or 10 lb. Cuprox per acre also maintained the copper content of the pasture above that of the controls for the three years of the observations. The effect of Cuprox was not as marked as that of bluestone when weight of copper applied is considered (Cuprox contains approximately twice the proportion of copper that bluestone does).

In the case of both the bluestone- and Cuprox-treated plots the level of copper in the pastures remained more or less constant for the last two years at the level that was reached twelve months after the single top-dressing. The results for these plots, considered along with results in Figs. 2A and 2B, show that the effect of a top-dressing with copper is to produce an immediate marked rise in copper content of the pasture, a rapid fall up to the end of the first twelve months, and then a persistent level of copper in the pasture higher than that in the untreated control areas.

*Gordonton Plots.*—Figure 3A shows results for plots 29, 30, 31, and 32 and the un-top-dressed area surrounding the plots. The first top-dressing of the plots was carried out on 15th February, 1944, and the graph prior to that time therefore shows the copper content of pasture samples from untreated plots. Top-dressing with bluestone at the rate of 2½, 5, 10, and 20 lb. per acre was carried out on 15th February, 1944, and 23rd March, 1945.

The Gordonton area differed from the Waitakaruru area in the higher content of copper in the untreated grass.



Only a very small increase in the copper content of the pastures was produced by a top-dressing of  $2\frac{1}{2}$  lb. bluestone per acre. Top-dressings of 5, 10, and 20 lb. per acre all caused an appreciable and approximately similar response. The difference between the effects of 5, 10, and 20 lb. per acre was not as pronounced on the Gordonton plots as on the Waitakaruru plots.

Figure 3B: This graph gives a comparison of the results from plots 5 and 8 and plots 13 and 16. Plots 5 and 13 were top-dressed in 1943 with 1 ton lime per acre, and bluestone, mixed with serpentine-superphosphate and muriate of potash, was applied in each of the three years 1943-45. Plots 8 and 16 were top-dressed with bluestone alone in 1943 and with bluestone mixed with 2 cwt. lime per acre in 1944 and 1945. Rates of bluestone application each year were 5 lb. per acre for plots 5 and 8 and 20 lb. per acre for plots 13 and 16.

Results for samples collected during the year after the first top-dressing are incomplete, but complete results are available for the remaining two years. In 1944 there was no appreciable difference when copper was applied with lime or when applied mixed with serpentine-superphosphate and potash. In 1945 bluestone mixed with lime produced a greater rise in copper content of the pasture than did the same amount of bluestone mixed with phosphate and potash.

Three successive annual top-dressings have not resulted in the copper content of the pastures reaching abnormally high values. There is a tendency for samples from all plots to be slightly higher in copper content over the third year than over the second year, but the levels reached were nowhere near the point at which stock health would be endangered.

Figure 3c: This graph compares plots 7 and 8 (5 lb. bluestone per acre) and plots 15 and 16 (20 lb. bluestone per acre); the first of each pair was top-dressed in the spring and the last of each pair top-dressed in the autumn. The results have been presented in the same manner as for the Waitakaruru plots by drawing the results for the spring top-dressed plots straight through and by transposing the results for the autumn top-dressed plots so that top-dressing dates lie on the same ordinate. The same symbols are used for plots top-dressed at the same rate, but the graph for the autumn top-dressed plots has large circles at intervals. In 1944 there was a marked effect in spring shortly after top-dressing and a rapid fall to values that were approximately similar to those for autumn top-dressed plots.

In 1945 there was very much smaller response to the spring top-dressing than to the autumn top-dressing.

The results from the Gordonton plots agree with those from the Waitakaruru plots in indicating a better response to top-dressing done in the autumn.

Figures 3d and 3e: These graphs show the persistence of effect from one top-dressing with bluestone or with Cuprox. In Fig. 3d are given the results for plots 6, 10, 14, and 20 top-dressed once on 2nd March, 1943, with, respectively, 5, 10, 20, and 40 lb. bluestone per acre, and in Fig. 3e results are given of plots 24 and 28 top-dressed once on 2nd March, 1943, with 5 and 10 lb. Cuprox per acre respectively.

In every case some effect was evident for the three years the observations were made. During the second year there was no significant difference between the residual effect from the different rates of top-dressing, but in the third year there was some segregation of results for the different rates and the copper content of pastures from the 5 lb. per acre plots was close

to that of the control areas. One interesting feature was that the autumn samples showed higher figures, indicating some tendency for a greater uptake of copper during that season from plots that had been once top-dressed.

*Wallaceville Plots.*—Figure 4: The purpose of the plots at Wallaceville was to determine whether dangerously high copper contents in pasture would be reached if copper was applied where there was no peat and where there was no evidence amongst stock of copper deficiency. The graph shows the results after two successive autumn top-dressings at the rates of 2½, 5, 10, and 20 lb. bluestone per acre.

There was an immediate response to each top-dressing, the content of copper in the pasture being higher the heavier the top-dressing with bluestone. In each year the effect of the copper top-dressing was of relatively short duration.

The effect of top-dressing with copper was, on the whole, less marked on Wallaceville soil than on the peat soils. There was no evidence in the two years of sufficient accumulation of copper in the soil to raise the copper content of the pasture to levels dangerous to stock.

*Top-dressed Fields.*—Figure 5A shows results of top-dressing fields adjacent to Waitakaruru plots.

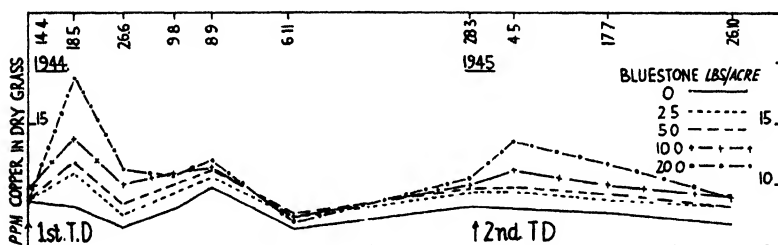


FIG. 4.—Wallaceville plots.

Section 50 was a single field, 52 acres in area, which was top-dressed in March, 1943, with 20 lb. Cuprox and 2 cwt. agricultural lime per acre; no further top-dressing was applied. Samples were taken at approximately monthly intervals until April, 1946.

The copper content of the pasture was exceptionally high for three months after top-dressing, the actual figures being 167, 185, and 105 p.p.m. copper. In the next three months the figures were 32, 55, and 30, and thereafter the figure was below 15 for the whole of the period of sampling. The unusually high figures for a period after sampling were probably not due to high absorption of copper from the soil. A more likely explanation is that these high figures were due to deposition of the finely ground Cuprox in the pasture during top-dressing and to the inclusion of some of the contaminated pasture at each sampling. This is probable, since there was a good growth of grass when top-dressing took place. Cuprox is insoluble in water and would not be readily washed off by rain, and the area was lightly stocked, having only 125 sheep on the 52 acres. The grass was not eaten off properly until later, when a large flock of wethers was employed. Small-scale experiments with as much as 50 lb. Cuprox per acre and in which the grass was mowed off each month after sampling have shown that it was only for the first sample that abnormally high figures were found.

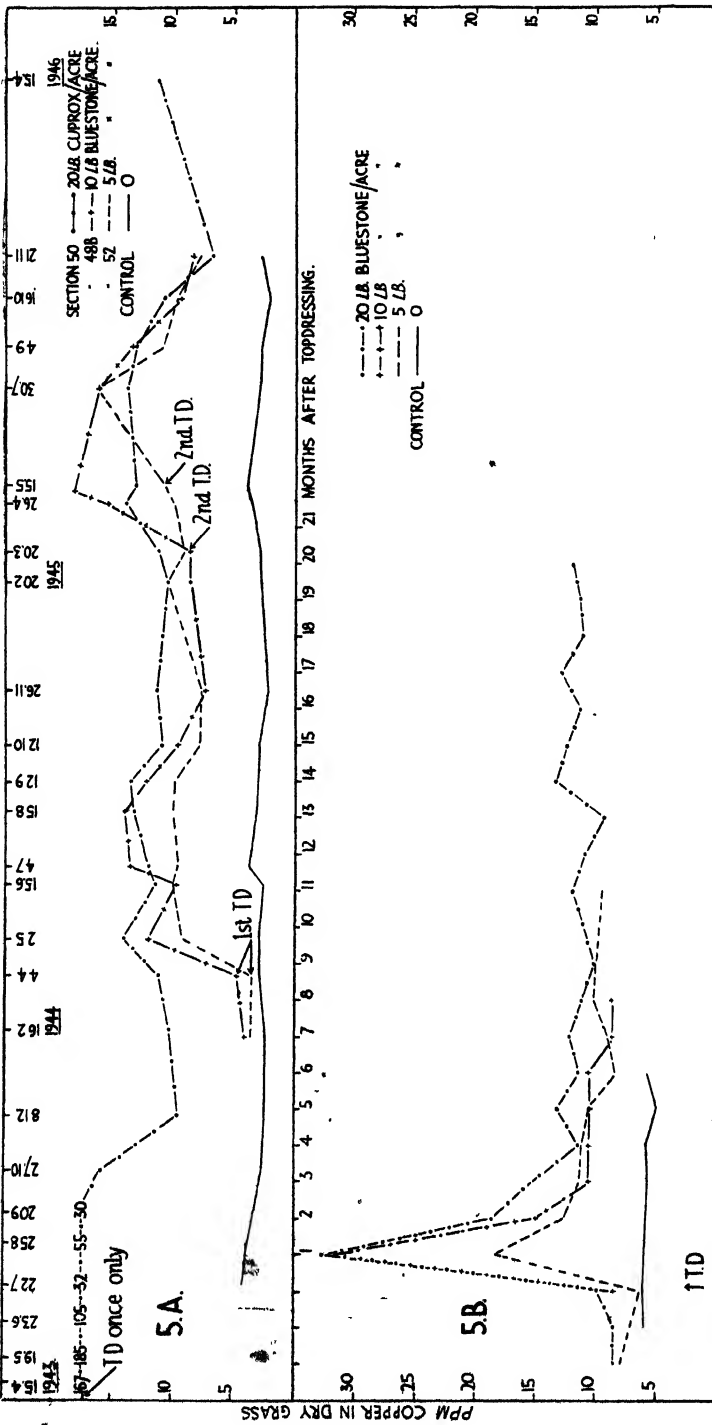


FIG. 5.—Top-dressed fields.

Whatever the explanation, however, it is of interest that the use of an insoluble form of copper in top-dressing, when the fertilizer is applied to long grass and the stocking is light, may result in stock ingesting abnormally high levels of copper for some months. That this can be dangerous to sheep was shown by deliberately leaving a number of animals on the area, thus exposing them to the risk of copper poisoning. Twelve out of twenty-five sheep died with symptoms of copper poisoning. After the original growth of grass was eaten down by a large flock of wethers no further trouble has been experienced in sheep that have remained continuously on the area for over two years.

The graph shows that one application of 20 lb. Cuprox per acre to Wai-takaruru soil is sufficient to maintain the copper content of the pasture at normal values for at least three years after the top-dressing.

Section 52, an area of 52 acres, was top-dressed in March, 1944, with 5 lb. bluestone in 2 cwt. agricultural lime per acre, and in May, 1945, with 2 cwt. copperized superphosphate, which supplied 5.6 lb. bluestone per acre. The first top-dressing raised the copper content of the pasture to approximately 10 p.p.m. at about which level it remained up to the second top-dressing, which caused a temporary rise. There was a subsequent fall again to approximately 10 p.p.m.

Section 48B, an area of 17 acres, was top-dressed in March, 1944, with 10 lb. bluestone and 2 cwt. lime per acre, and again with the same quantities in March, 1945. The first top-dressing produced a rise in the copper content of the pasture which was sustained until the second top-dressing took place, and then there was a still further increase in pasture copper, with a subsequent fall to values approaching 10 p.p.m.

The results of Sections 52 and 48B agree reasonably closely with those obtained in the plot experiments (see Figs. 2A and 2B) in showing that top-dressing with 5 or 10 lb. bluestone per acre raises the copper content of the pastures to normal values. The agreement is of further significance in showing that the detailed observations made on the plots can safely be employed as a guide for paddock-scale work.

Ewes kept on these top-dressed areas have produced normal lambs, whereas there has been up to 100 per cent. incidence of enzootic ataxia in lambs from comparable ewes on adjacent untreated areas.

Figure 5B: This graph summarizes the effect on the copper content of pasture of top-dressing carried out on three dairy-farms as a prophylactic measure against peat scours. Most of the fields on each farm were top-dressed at the rate of 5 lb. bluestone and 2 cwt. lime per acre; on two farms one field was top-dressed at the rate of 10 lb. bluestone per acre; and on each of the farms one field was top-dressed at the rate of 20 lb. bluestone per acre. The graphs shown are mean results for each rate of top-dressing and for un-top-dressed areas on the same farms. Observations on 5 and 10 lb. per acre areas do not extend beyond the first year. No further top-dressing was applied to the 20 lb. per acre areas for two years and observations were continued for this time. The results show that all these levels of top-dressing raise the copper content of the pasture appreciably above that of the untreated areas.

For two to three months after top-dressing there was a higher content of copper in pastures top-dressed with 10 and 20 lb. bluestone per acre than in pastures top-dressed with 5 lb. bluestone per acre. After the third month, however, the results for each rate were substantially the same.

The copper content of pastures top-dressed with 5 and 10 lb. bluestone per acre was maintained at approximately 10 p.p.m. for ten months after top-dressing (the whole of the period of observation), and the copper content of pastures top-dressed with 20 lb. bluestone per acre remained slightly above 10 p.p.m. for twenty months after top-dressing. These results resemble fairly closely those obtained on plot experiments on similar farms.

In years prior to top-dressing, all dairy stock on these farms were subject to peat scours in acute form. The effect of the top-dressing was to banish the scouring symptoms and to restore the health and production of the stock to normal. This result could be attributed to the use of 5 lb. bluestone per acre, as this was the rate of top-dressing for more than 95 per cent. of the area on each farm.

*Effect of Top-dressing with Copper on the Copper Content of Hay.*—Some samples were taken of hay made from fields that had been top-dressed in the spring after shutting up for hay. The results obtained are shown in Table I, which also gives the available results for un-top-dressed hays from the same farms. The hay-fields which were top-dressed had not had copper applied in any form before and the un-top-dressed hay-fields had not at any time been top-dressed with copper compounds.

TABLE I.—COPPER CONTENT OF HAY TOP-DRESSED WITH BLUESTONE (P.P.M. CU IN DRY MATTER)

Farm No.		Top-dressed Hay.			Mean.	Un-top-dressed Hay.			Mean.
1	..	5.7	7.8	13.8	9.1	..	..	..	..
2	..	5.7	7.5	..	6.6	3.9	5.1	5.8	4.9
3	..	4.9	..	..	4.9	4.1	..	..	4.1
4	..	11.6	..	..	11.6	4.1	..	..	4.1
5	..	6.3	6.7	7.7	6.9	4.3	..	..	4.3
Mean ..		..	7.8	..	..	..	4.6	..	..

The results show considerable variation in the effect of top-dressing with bluestone. In some cases there was very little increase in the copper content of the hay, whereas in others a marked effect was produced. The mean results for ten top-dressed hays was substantially higher than the mean for six un-top-dressed hays, which would suggest that, on the whole, the practice of top-dressing hay crops would increase the copper supplied to stock in this fodder.

*Effect of Top-dressing with Copper on Botanical Composition of the Herbage.*—Observations were made to determine whether the application of copper compounds as fertilizers had any effect on the botanical composition of the pastures.

It has already been stated that the pasture on some of the farms where peat scours occurred contained about 60 per cent. of Yorkshire fog, with perennial rye-grass, white clover, and sometimes paspalum and other grasses making up the remainder of the pasture. On other "peat scours" farms the pasture was predominantly rye-grass and white clover. Repeated top-dressings for two or three successive years have not materially altered the botanical composition of these pastures. On one farm where, in the past, newly sown rye-grass very rapidly gave place to Yorkshire fog, there has been a tendency, after top-dressing with copper, to maintain a greater proportion of rye-grass for long periods. Circumstances made it possible for only a very few observations of this effect and the results cannot therefore be very confidently stated.

On the Waitakaruru area a very definite effect of copper was manifested. Prior to top-dressing, these areas had a pasture composed almost entirely of Yorkshire fog and *Lotus major*. The area top-dressed with 20 lb. Cuprox and 2 cwt. lime (see Fig. 5A) and the area top-dressed with 5 lb. bluestone in 2 cwt. lime one year and 5.6 lb. bluestone in 2 cwt. superphosphate in the second year now contain approximately 40 per cent. of perennial ryegrass and white clover. The improvement in this area in contrast to the lack of effect on the areas discussed in the previous paragraph may be associated with the fact that Waitakaruru was much more deficient in copper, the average content in untreated pasture being 3.6 p.p.m., compared with 7.1 p.p.m. from "peat scours" areas.

Another feature at Waitakaruru was the establishment of the common Scotch thistle (*Cirsium lanceolatum*) on top-dressed areas. This was particularly noticeable, as top-dressed areas grew a heavy crop of this thistle, whilst completely surrounded with un-top-dressed areas growing very little thistle. A similar phenomenon was noted on the plots, where there was on adjacent small plots a differential rate of establishment of thistles. This varied directly in proportion to the amount of copper that had been applied to the plots. A count of the thistle population was made, and the figures, together with the rate of copper top-dressing, are shown in Table II.

TABLE II.—COMPARISON OF THISTLE ESTABLISHMENT AND RATE OF COPPER TOP-DRESSING

Copper Top-dressing (Pounds per Acre)				Number of Thistle Plants per 250 Acres.
Nil	..	..	..	1
5 Bluestone	..	..	..	5
10	"	..	..	7
20	"	..	..	15
40	"	..	..	17
5 Cuprox	..	..	..	8
10	"	..	..	15

The figures for each  $\frac{1}{250}$  acre in Table II are averages of a number of plots (in most cases eight) top-dressed in the same manner. Top-dressing was carried out ten months earlier and the thistle counts were made in February, 1944.

The phenomenon did not occur in the autumn of 1945 or 1946.

The only explanation that has been advanced by botanist colleagues is that the thistle is a plant which requires high fertility in the soil and that the provision of copper supplied an element necessary for its establishment. Analyses of thistle in the rosette and seeding stage collected from an area not deficient in copper show that they contain approximately one and a half times the copper content of pastures growing on the same area. It seems probable, therefore, that a high storage requirement for copper does not explain the readier establishment on top-dressed areas. The appearance of thistle on developing peat land is regarded favourably by practical men and taken as an indication that the land is reaching a stage in its development that is suitable for sowing better grass mixtures.

The use of copper as a fertilizer, then, does not greatly affect the nature of the pasture plants on peat land that is only moderately deficient in copper. On more deficient land the establishment of perennial ryegrass and white clover is favoured, and shortly after the fertilizer has been applied there is sometimes a heavy growth of Scotch thistles (*Cirsium lanceolatum*).

## DISCUSSION

The results given above show that the use of copper compounds as fertilizers causes an increase in the copper content of pasture grown on peat soils or on clay soils. This response occurs when bluestone, a salt of copper soluble in water, or when Cuprox, an insoluble salt, is employed. Most of the work has been concentrated on bluestone, as this is the cheapest and most readily available source of copper in New Zealand. Much of the work was carried out on small plots  $\frac{1}{16}$  acre in area, but the general validity of these results has been established by their similarity to results obtained from fields top-dressed at the same rates. It is, in fact, true that the plots indicate more faithfully the effect of copper when it becomes washed into the soil, since the plots were closely grazed after each sampling and therefore the copper content of successive pasture samples represents the copper that has been taken up by the plant in making new growth. Samples collected from fields, especially those lightly grazed, may be affected for some months by copper which adhered to the plant when the top-dressing was applied.

The results show that a response is elicited by as little as  $2\frac{1}{2}$  lb. bluestone per acre, but that 5 lb. per acre raises the level of pasture copper to approximately normal values on all the deficient areas studied. There is no appreciable difference when bluestone is applied in conjunction with agricultural lime and when applied with lime, superphosphate, and potash.

The importance of the results is in the bearing they have on the control of copper-deficiency diseases in stock kept on peat lands(4, 5). Other work has shown that these diseases are controlled when the copper content of the pastures is brought to normal values—i.e. in the region of 10 to 12 p.p.m. of copper in the dry grass. The results from different rates of top-dressing show that this is effected best by the use of 5 lb. bluestone per acre. The absence of any major difference when bluestone is applied with other fertilizers means that the normal routine of farm top-dressing need not be greatly disturbed in employing copper as a fertilizer. It can be applied mixed with agricultural lime or with superphosphate or even alone. Its successful use with superphosphate has led to the manufacture of a ready mixed copperized superphosphate the use of which avoids the labour of mixing on the farm. The fact, too, that autumn top-dressing has a better effect in sustaining the level of copper in the pasture is most suitable so far as farm practice in New Zealand is concerned, since most top-dressing is carried out in the autumn.

From the fact that higher amounts of bluestone—in particular, 20 lb. per acre—exert an effect that persists for two years or more, it might be argued that it would be more economic of labour to employ this means of restoring the copper status of the soil to normal. It is felt by the authors, however, that a routine should be established in which a certain correct amount of copper is applied each year. Establishment of an annual routine ensures that copper will be applied; a plan which provides for top-dressing every three years might not be faithfully adhered to and might lead to forgetfulness of the time for a fresh top-dressing. This is important, as the stock-diseases which it is the purpose of the copper to prevent are insidious in onset and there may be considerable loss of production and deterioration in health before the diseases become clinically recognizable. Correctness of amount implies the minimum, and thus the cheapest quantity which will maintain the copper content of the pastures at normal levels and which will not lead to accumulation of copper in the soil, with the attendant risk of high copper content of pastures. An annual rate of 5 lb. copper sulphate per acre fulfils all these requirements. It has been shown in practice to be

effective in preventing stock-diseases, it is cheap, and there appears to be no danger of copper accumulation in the soil. Justification for the last statement is derived from the fact that there is no evidence of any such accumulation after the third annual top-dressing with 20 lb. per acre. The inference is that 5 lb. per acre would be safe for many more years. This question of possible accumulation is still under investigation and areas will be top-dressed annually for some years in order to determine whether any danger can occur.

It is of some interest that similar amounts of copper fertilizers have a more persistent effect on peat soils that are deficient in copper than on a clay soil that is normal in copper content. The small effect that was produced on the Wallaceville clay is of importance in extension work regarding the use of copper for top-dressing. It indicates that no dangerous amount of copper is likely to occur in pastures grown on soils normal in copper content and which are mistakenly treated with copper fertilizers.

Top-dressing hay-fields with bluestone was shown to increase the copper content of the hay to a small extent. Hay from copper-deficient country is lower in copper content than hay from normal country. It is of some importance that a normal amount of copper should be supplied in the hay. The developing foetus makes its final growth in the hay-feeding season, and the mother also should build up a reserve of copper to prepare her for the spring, when the tendency for the occurrence of peat scours is greatest.

The use of copper-containing fertilizers has been shown to increase the copper content of deficient pastures to amounts that are sufficient for the needs of farm stock. The question of the effect on plant growth was also considered. The copper fertilizers did not affect the herbage in any adverse manner. Copper sulphate mixed with lime or other fertilizers had no harmful effect on pasture: even copper sulphate alone had no harmful effect when applied in small quantities, though a rate of 50 lb. per acre did produce a negligible amount of spotting or burning on the leaves of plants. Subsequent growth, however, was not affected. There was, on the other hand, a favourable influence of copper on the establishment of better grasses such as perennial rye-grass and white clover on very deficient land. This effect was not marked on less-deficient land, and on such land did not extend beyond prolonging the period during which newly sown rye-grass resisted replacement by Yorkshire fog. No detailed study of the effect of copper on species composition of pastures was attempted, but the observations made are commented upon, as they indicate the possible value of copper fertilizers in developing copper-deficient lands.

#### ACKNOWLEDGMENT

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## THE POLAROGRAPHIC ESTIMATION OF ASCORBIC ACID IN MILK

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### Summary

The polarograph can be used for the estimation of ascorbic acid in solution by measurement of the height of the diffusion-current curve. For solutions of low ascorbic acid content, such as milk, the addition of a small amount of a copper salt provides a reference curve, the measured differences between the two curves being the "step-height" of the ascorbic acid present in the solution. Comparative experiments with the titration method show satisfactory agreement. The technique described is probably applicable to other biological fluids.

### INTRODUCTION

THE polarograph may be used for the estimation of substances that are electro-oxidizable or -reducible in solution. The substance to be determined must be in true solution and is electrolyzed between two electrodes, one of which consists usually of fine drops of mercury falling from a capillary placed in the solution. When a gradually increasing voltage is applied to the electrodes and the resulting current-voltage curve is plotted, a typical curve, described generally as a "step," is obtained. This method has been applied to the determination of many organic substances containing groups capable of oxidation or reduction.

Ascorbic acid, in acid solutions, undergoes electro-oxidation, giving a well-defined diffusion-current curve. It was therefore thought worth while to attempt the polarographic determination of ascorbic acid in milk, to ascertain whether the results obtained would be comparable with the values yielded by a titration procedure such as that of Kon and Watson(6) or of Harris and Olliver(3). In brief, this method consists of precipitation of the milk proteins, and titration of a portion of the filtrate with sodium 2:6 dichlorophenol-indophenol solution. A recent article(2) describes the polarographic estimation of ascorbic acid in vegetables and fruit juices, but the level of ascorbic acid in milk is very much lower than in these materials, so that the technique described is not immediately applicable. Also, the chloride-ion concentration in milk is such that the chloride step interferes over the range of pH used for vegetables and fruit. Because hydrogen-ion is a component of the electrode reaction, the ascorbic acid step depends on the pH of the solution, the relation being  $E_1 = E_0 - 0.06 \text{ pH}$ , and it is possible to avoid the chloride step by having the pH of the solution above about 5.6. However, with almost all buffers the oxygen step would then interfere, necessitating removal of air, but, for a reason not yet understood, potassium hydrogen phthalate-sodium hydroxide buffers displace this wave to more negative values, and we have found that reproducible steps can be obtained for ascorbic acid by operating with the pH of the milk solution adjusted to 6.0, the molarity of phthalate in the final solution being 0.05. A detailed description of the methods established and recommended is given below.

### INSTRUMENT

In view of the simplicity and cheapness of the manual polarograph, a brief description is given of the instrument constructed in this laboratory and used for the ascorbic acid estimations. All polarographic circuits are fundamentally similar, and consist of a continuously variable potentiometer to supply the voltage across the electrodes, together with a current measuring device, usually a long-period galvanometer, in series with the electrodes. Figure 1 is largely self-explanatory. For  $R_3$ ,  $R_4$ , and  $R_5$

equal lengths of constantin wire are cut;  $R_8$  is also wound from this wire, but a finely wound radio potentiometer would be satisfactory. The initial adjustment of the apparatus is made by setting  $R_1$  and  $R_2$  so that the voltmeter gives full deflection when the voltage across  $R_4$  is 1.000 as measured by the millivolt scale of a pH meter. In subsequent operation  $R_2$  is fixed and  $R_1$  is adjusted as required to keep the voltmeter set to full-scale reading. A dial fitted to  $R_4$  is then calibrated using a pH meter to read from 0 to 1.000 volts. The voltages across  $R_3$  and  $R_5$  are measured in the same manner, the former being 0.980 volts because of the parallel circuit through the voltmeter and  $R_2$ . The galvanometer has a period of

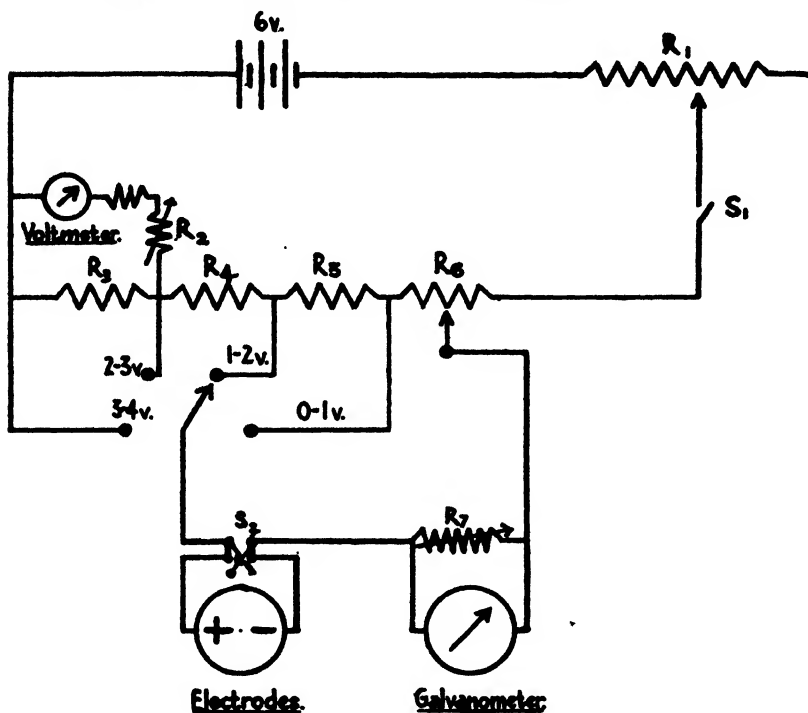


FIG. 1

Voltmeter	0-500 micro-ammeter.
Galvanometer	Leeds and Northrup, type N.
$R_1$	0-100 ohm variable resistance.
$R_2$	0-2,000 ohm variable resistance.
$R_3, R_4, R_5$	Matched, 20 ohm resistances.
$R_6$	Slightly greater than 20 ohm, variable resistance.
$R_7$	0-2,000 ohm variable resistance.
$R_8$	Switch.
$S_1$	Switch.
$S_2$	Polarity-reversing switch.

three seconds and a sensitivity of 0.0026 microamp. per millimeter, but is slightly overdamped to decrease the oscillation of the light beam during the growth of the mercury drops. The capillary electrode has been constructed by drawing out a piece of fine thermometer tubing, its characteristics being  $m \frac{2}{3} t \frac{1}{3} = 1.63$ , where  $m$  is the mass of mercury falling in milligrams per second, and  $t$ , the drop-time, = 2.00, the measurements being made on open circuit at 18° c. in 0.1 N-potassium chloride solution saturated with air. Corrected for back pressure the height of the mercury column used is 109 cm. Using this electrode, the step height which has been found to be linear with concentration is approximately 0.080 microamp. for 1.0 mg. of ascorbic acid in 100 ml. of milk at  $18 \pm 0.5^\circ$  c.

## POLAROGRAPHIC METHOD

A buffer solution is prepared by adding 14 ml. of 1.30 N-sodium hydroxide to 100 ml. of 0.2 N-potassium hydrogen phthalate saturated with thymol as preservative. To 10 ml. of whole milk in a 30 ml. beaker is added 4 ml. of this buffer, and the solution stirred to ensure thorough mixing. The beaker is placed in a bath at 18° C. and the dropping electrode and a potassium nitrate-agar salt bridge are immersed in the liquid. The reference electrode is saturated calomel. Mean readings are taken at  $-0.02$ ,  $+0.07$ , and  $+0.08$  volts. The reading at  $-0.02$  volts is subtracted from the average of the readings at  $+0.07$  and  $+0.08$  volts to give the difference,  $d$ . Readings at both  $0.07$  and  $0.08$  volts are taken in order to diminish experimental error; for routine purposes either reading would probably be sufficiently accurate. After adding 0.2 ml. of 0.4 per cent. copper sulphate solution to the milk-buffer mixture, it is stirred and left for several minutes, the ascorbic acid being completely destroyed within five minutes. The readings are then repeated and the difference,  $d$ , obtained as before. The change in this value gives the step height of the ascorbic acid curve, so that the ascorbic acid concentration of the original milk sample can be read from the standard graph, which is prepared by adding known amounts of ascorbic acid to milk and measuring the resulting change in  $d$ . A small "blank" of 0.07 mg. ascorbic acid per 100 ml. has to be subtracted from this value to allow for the effect on the residual current of the addition of the copper sulphate solution. The magnitude of this correction has been confirmed both by extrapolation using varying amounts of copper, and by irradiation in sunlight.

It is necessary to take care that the dropping electrode remains clean, otherwise dropping becomes irregular and faulty readings are obtained. A warm solution of sodium metasilicate has been found to be efficient for this purpose, the electrode being cleaned after about six samples have been analysed, and rinsed with water between samples.

## TITRATION METHOD

Since the estimation of ascorbic acid in milk is usually done by titration, the results obtained by this new polarographic method have been compared with those given by titration with 2:6 dichlorophenol-indophenol. A solution containing 0.01 mg. per millilitre of the sodium derivative of this dye is made up, and when not in use this is stored in a refrigerator (40° F. to 45° F.). A fresh solution should be prepared every eight to ten days. Even when kept in a refrigerator, the dye solution decomposes slowly, so its strength must be checked daily. The direct method consists of titration against a weighed small amount of ascorbic acid (the purity of which has been checked previously by titration against 0.01 N-iodine solution). It has been found that this procedure for standardizing gives good agreement with the iodometric methods using 0.01 N-sodium thio-sulphate(4, 5), and also with the method using Mohr's salt (ferrous ammonium sulphate), providing the precautions specified by Stewart and Sharp(8) are carefully observed. The strength of the dye solution is calculated in milligrams of ascorbic acid per millilitre of dye solution, enabling the ascorbic acid concentration of the sample to be readily assessed. Owing to the difficulties of directly weighing and titrating very small amounts of ascorbic acid, Stewart and Sharp's method is preferable, especially as its end point is similar to that obtained in milk filtrates.

For preparing a protein-free filtrate from milk, a solution of metaphosphoric acid is preferred, as it appears to have a stabilizing effect which prevents oxidation of the ascorbic acid(1). The 10 per cent. solution must be prepared each week and stored in a refrigerator. The procedure

for preparing the filtrate is as follows: to 15 ml. of 10 per cent. metaphosphoric acid is added 15 ml. of whole milk. After thorough mixing and allowing to stand for five minutes, the mixture is filtered through a dry Whatman No. 1 filter paper (9 cm.), the initial portion of the filtrate, if cloudy, being passed once more through the same filter paper. Duplicate 10 ml. portions of the filtrate are titrated against the dye solution, using a 2 ml. micro-burette, until the pink colour obtained persists for thirty seconds—that is, till rapid fading ceases. The titration value is corrected by subtracting a blank value.

### RESULTS

To test the reproducibility of the methods used, replicate analyses for ascorbic acid were performed on a series of milk samples. The results obtained, as milligrams of ascorbic acid per 100 ml. of milk, are given in Table I. The results by either method appear to be reproducible to approximately  $\pm 0.1$  mg. For all except two of the samples, agreement between the two methods is very satisfactory, and in these two cases (Nos. 2 and 4) the titration procedure gives significantly higher results. This may be due to the lack of specificity of the indophenol dye method, since this method is known to be unable to distinguish ascorbic acid and certain other reducing substances which may be present in some milks.

TABLE I.—COMPARISON OF TITRATION AND POLAROGRAPHIC RESULTS  
(Ascorbic acid expressed as milligrams per 100 ml. of milk)

Description of Sample.	Titration Method.	Average.	Polarograph Method	Average.
1. Raw milk, source A, as available to consumer—				
(a) .. .. .	0.33	0.39	0.31	0.38
(b) .. .. .	0.33	0.33	0.25	0.26
	0.33		0.21	
Same as (b), plus 10 p.p.m. added ascorbic acid	..	1.30	..	1.30
Same as (b), plus 20 p.p.m. added ascorbic acid	2.35 2.39	2.37	2.42 2.41 2.41	2.41
2. Raw milk, source B, as available to consumer	1.91 2.03 2.01 2.05	2.00	1.62 1.64 1.70	1.65
Same sample after two hours' exposure to sunlight on dull day	0.15	0.16	0.26	0.26
Same sample after thirty-six hours at 4° C.	0.17		0.26	
	..	1.45	..	1.48
3. Pasteurized milk, as available to consumer	0.30 0.36 0.37 0.36 0.35 0.31	0.34	0.37 0.46 0.26	0.36
Same sample after seventy-two hours at 4° C.—				
(a) .. .. .	..	0.10	..	0.15
(b) .. .. .	..	0.13	..	0.11
4. Individual cow sample after forty-eight hours at 4° C.	0.60 0.57 0.58 0.55	0.58	0.34 0.36 0.20	0.30

The ascorbic acid content of milk from twenty-five individual cows was determined by the titration procedure within three hours of milking. The results obtained are included in Table II. Unfortunately, it was not possible to perform the polarographic analyses at the same time, but these and a further series of titrations were carried out after the samples had been stored in brown bottles for forty-eight hours at 4° C. The values given in Table II are of single determinations by both methods. Agreement between the two procedures is generally satisfactory, within the variability of the methods. The error in the polarographic method may perhaps be increased slightly by the ageing of the milk samples, which is accompanied by solidification of the fat globules. It is of interest to record that the initial ascorbic acid content ranged from 1.03 to 2.21 mg., with a mean value of 1.76 mg. per 100 ml. of milk, which is very close to the average value reported for bulk raw milk by Stewart and Sharp(9). After storage in the dark for forty-eight hours at 4° C., the ascorbic acid content dropped appreciably, ranging from 0.41 to 1.74 mg., with a mean value of 1.16 mg. per 100 ml. of milk.

TABLE II.—ASCORBIC ACID CONTENT AS MILLIGRAMS PER 100 ML. OF MILK FROM INDIVIDUAL COWS

Cow.	Within Three Hours of Milking. Titration Method.	After Forty-eight Hours at 4° C.	
		Titration Method.	Polarographic Method.
Ada ..	1.95	1.21	1.12
Becta ..	1.77	1.12	0.99
Chloe ..	1.66	1.16	1.27
Dawn ..	1.93	1.54	1.40
Dinny ..	2.06	1.71	1.69
Hope ..	1.03	0.46	0.53
Sis ..	1.73	1.50	1.35
Tina ..	2.20	1.73	1.49
2 ..	2.21	1.38	1.57
7 ..	1.73	1.24	1.35
8 ..	2.15	1.58	1.90
11 ..	2.03	1.44	1.51
12 ..	1.56	0.53	0.38
13 ..	1.73	1.49	1.65
14 ..	1.53	1.24	1.49
16 ..	1.24	0.26	0.56
E1 ..	1.46	0.58	0.30
E2 ..	2.17	1.16	1.07
E3 ..	1.44	1.12	0.98
E4 ..	1.71	1.31	1.29
E5 ..	1.49	0.80	0.69
E7 ..	1.72	1.09	1.49
E9 ..	1.29	0.71	0.51
E11 ..	1.68	1.12	0.75
F1 ..	2.12	1.57	1.50

An increase in ascorbic acid content as determined by titration has been observed when milk samples are laboratory-pasteurized. To establish whether this effect was real or due to the production of interfering substances, a small set of samples, heat-treated in various ways, was examined by both methods, giving the results shown in Table III. Values by both methods

agree within the limits of error. The increments resulting from heat-treatment appear to be significant and are probably due to reduction of dehydro-ascorbic acid present, effected by reducing substances—*e.g.*, sulphides—produced by the action of heat on milk proteins.

TABLE III.—HEAT-TREATED MILKS

Method of Heating.	Titration Method.	Polarographic Method
Bulk raw milk, unheated .. .. .	0.97	0.73
Laboratory-pasteurized by holding at 145° F. to 150° F. for thirty minutes	0.88	0.85
Laboratory-pasteurized by flash process: heating to 165° F. to 170° F. for thirty seconds	1.20	1.03
Laboratory-sterilized by standing in boiling-water bath for twenty minutes	1.31	1.36

The actual level of ascorbic acid content of milk from each individual cow was found in most cases to be fairly constant over a period of several weeks, in which the samples were taken at intervals of a few days. This was observed using both polarographic and titration procedures, as indicated in Table IV. As these results were obtained from the milk of cows which were in the same herd and which were sampled for analysis in the late summer when ample green pasture was available, the level of ascorbic acid in the milk would appear to be an individual peculiarity of each cow. The levels are similar in range to those reported by Ray *et al*(7).

TABLE IV.—ASCORBIC ACID IN MILK FROM INDIVIDUAL COWS, REPEAT SAMPLES

Cow	Ascorbic Acid, as Milligrams per 100 ml. of Milk
Tina ..	2.10, 2.10, 2.07, 2.14, 2.10, 2.13, 2.20.
Dinny ..	2.15, 2.02, 2.06.
11 ..	2.00, 2.08, 2.03.
2 ..	1.79, 1.87, 2.00, 2.03, 2.21.
Chloe ..	1.63, 1.86, 1.75, 1.66.
E1 ..	1.47, 1.41, 1.50, 1.46.
12 ..	1.49, 1.42, 1.56.
16 ..	1.11, 1.29, 1.27, 1.41, 1.24.

## DISCUSSION

Although the present investigation has been limited to ascorbic acid, as distinct from dehydro-ascorbic acid, there does not appear to be any reason why the polarographic method should not be employed to measure "total" ascorbic acid, by regeneration from dehydro-ascorbic acid, either by the bacteriological method using *B. coli*, or by the chemical method with hydrogen sulphide (as may have happened to provide the results in Table III). The former method would have the advantage of being considerably more rapid than the latter, an important consideration with so unstable a substance as ascorbic acid, and an effort will be made to apply this procedure as soon as a sufficiently active bacteriological culture is available.

For the estimation of ascorbic acid the polarographic method described here has several advantages over the titration method. The frequent preparation and standardizing of solutions is avoided once the calibration curve has been constructed, while the buffer solution which is used is stable. There is also reason to believe that the polarographic method is more specific.

The use of added copper to provide a reference curve for the polarographic estimation of ascorbic acid appears to be original. Such a procedure is necessary because the ascorbic acid concentration is too low to be measured directly as a specific step on the curve for whole milk, and also because milk contains other substances giving polarographic steps over the range of voltage used. The addition of copper destroys the ascorbic acid and thus permits a second reading; the difference between this and the first reading gives a measure of the ascorbic acid. For a substance to interfere in the determination of ascorbic acid by the present method it must fulfil two conditions—first, part of its “step” should occur over the range of voltage used, and, second, it should either be destroyed by, or form an insoluble complex with, copper. With the exception of ascorbic acid, no substance fulfilling these conditions and present in milk is known to the authors, so the method appears to be specific for this vitamin. The most likely interfering substance is cysteine, but experiments have shown that this compound does not interfere. For example, in trials using 0.6 mg. of cysteine per millilitre of buffered solution the cysteine step was found to be affected by the addition of copper to the solution. The close agreement of results given by the polarographic and the titration method is further evidence that there are no interfering substances.

In solutions which are strongly coloured, ascorbic acid cannot be determined accurately by the use of the sodium 2:6 dichlorophenol-indophenol dye titration method. Further, many biological fluids are known to yield false values, due to interfering substances which also reduce the indophenol dye. This is due to the inability of the indophenol method to distinguish accurately between a number of oxidation-reduction systems which include that of ascorbic acid. All systems with a redox potential equal to or less than that of the ascorbic acid - dehydro-ascorbic acid system are included in titration or colorimetric methods which use sodium 2:6 dichlorophenol-indophenol. There is good reason to expect that, with very slight modifications, the polarographic technique described could be adapted for the estimation of ascorbic acid in biological fluids, such as those referred to, where the indophenol dye titration method is unreliable. Polarograms have been reported in the literature using volumes of less than 0.05 ml., so that an additional advantage of this method is the small sample required. This is because the “step” height is dependent only on the concentration of ascorbic acid and not on the volume of solution.

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## THE ESTIMATION OF COPPER IN CREAM

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### Summary

To enable the determination of copper in cream, the filtration method devised for butter has been suitably modified. Improvements have also been made in the wet-ashing method. The technique for applying these methods to cream samples is described in detail and figures are given showing close agreement between the two methods. Tables of results are given to demonstrate the value of these methods for enabling copper contamination of cream and butter to be detected and eliminated.

### INTRODUCTION

IN 1940, details of a new filtration method for estimating the copper content of butter were published(6), together with evidence that the results which it yielded were in good agreement with those obtained by a modified wet-digestion method. The success achieved with this simplified filtration method led to a desire to apply it to the determination of copper in cream. From time to time Dairy Instructors have been in doubt about the precise origin of excessive amounts of copper found in butter, so that analyses of cream for copper content were necessary to provide them with further information. In a butter-factory, contamination with iron does not often occur, but copper readily gets into the cream from untinned copper and brass fittings. The estimation of copper in cream was therefore given first attention, while the determination of iron was left for later study. The methods which have been established after much experimental work are set out below.

### DAIRY FACTORY LAYOUT

To enable details of sampling points for cream to be better understood, a brief description of the layout and operation of butter-factory machinery seems worth while. On arrival at the factory the cans of cream, after grading and weighing, are tipped into neutralizing-vats having a capacity of 200 to 600 gallons. The neutralized cream is pumped through the pasteurizing-machine, and thence to the top of the coolers, over which it gravitates. From the bottom of the coolers it is again pumped into refrigerated vats, where it is kept cold overnight. Next morning the cream in the vats is stirred and pumped into the churns.

The copper content of the cream on arrival at the factory is usually quite low (0.10 to 0.15 p.p.m.). A little of this may come from untinned brass piping about the milking-machines, or from copper coolers the tinning of which has become worn, but the use of tinned steel cans does not increase the amount in transit. If tinned copper neutralizing-vats are in use, bare copper is often visible; the tin coating may be worn off parts of tinned copper coolers and distributing-trays, while brass piping, and especially pumps, often show substantial untinned portions.



The refrigerated holding-vats formerly used for keeping the cream at a temperature of 40° F. to 50° F. overnight were of the long rectangular type with D-shaped cross-section made of tinned copper, from which cleaning operations frequently abraded some of the tin coating. During the last ten years in many of the largest factories, as well as in some smaller ones, these D-shaped vats have been replaced by tall cylindrical vats of stainless steel. The D-shaped vats are still retained in some cases as a standby or for holding lower-grade cream. Many factories still use the two-stage pasteurizer of tinned copper, through which the cream is forced by rotating beaters, but in others a new type of machine known as the vacreator has recently been installed. This is made almost entirely of stainless steel. Other equipment of stainless steel for coolers and piping has been introduced to a more limited extent, so that the opportunity for contamination of cream with copper is now appreciably less than formerly. Nevertheless, examples can be quoted to show that even when a factory plant is almost entirely made up of stainless steel very serious copper contamination can still occur.

## DESCRIPTION OF METHODS

### *Sampling*

For procuring samples of cream from dairy factories which have persistently shown high figures for copper in their butter, 8 oz. to 10 oz. bottles are specially prepared by treating them with chromic or nitric acid, followed by rinsing with glass-distilled water. Rubber stoppers for these bottles are soaked for an hour or two in dilute (5 per cent.) nitric acid. To each bottle is added 1 ml. of 35 per cent. Analar formaldehyde solution as a preservative. So that a clue may be obtained as to the source of the copper contamination, these samples are taken at certain definite points—e.g., after the pasteurizers, after the coolers, from the delivery pipe to the cold vat or to the churn. Samples of cream are sometimes rather variable in composition, due to added water. Prior to running the cream through the pasteurizers it is customary to set the machinery in motion and run water through the plant in order to attain the desired pasteurizing temperature. This water circulates over the plant, so that the first sample obtained at the beginning of operations may contain only 5 per cent. to 10 per cent. of fat. Succeeding samples taken at the same point become progressively richer until finally whole cream is obtained (about 40 per cent. fat). At the end of the pasteurizing operations a certain amount of water is also used to rinse cream off the machinery, and this is usually run into the cold vat. Consequently, samples obtained the following day from the tap at the bottom of a tall holding-vat, before its contents have been stirred, may be quite watery with little fat, but with appreciable amounts of milk protein. Thus any method of analysis for copper in cream must be generally applicable to these various types of samples.

### *Reagents required*

Glass-distilled water is necessary for washing apparatus and for making up solutions. All glassware, after thorough cleaning, is rinsed with nitric or chromic acids, followed by glass-distilled water. To reduce the blanks to a minimum, the principal reagents, except sulphuric acid and hydrogen peroxide, are purified. By testing available supplies, the purest ammonia, having a copper content less than 0.05 µg per millilitre,

is selected. During the preparation of reagents and at all later stages care must be taken to ensure protection from contamination by dust particles, which may include minute fragments of copper.

*Hydrochloric Acid* (C.B.—i.e., approximately Constant-boiling Point).—Prior to distilling, commercial hydrochloric acid should first be diluted with water to specific gravity 1.12, a little higher than the figure for C.B. acid.

*Nitric Acid* (Concentrated and 5 per cent. Solution).—Prepared by distillation of commercial nitric acid, using an all-glass still.

*Sulphuric Acid*, concentrated, Analar.

*Trichloroacetic Acid* (20 per cent. and 5 per cent. Solutions).—Made from acid which has been distilled *in vacuo*.

*Hydrogen Peroxide*, 30 per cent. solution.

*Sodium Tungstate* (10 per cent. Solution).—Purified by adding a few milligrams of sodium diethyl-dithio-carbamate followed by three or four extractions with amyl acetate in a separating funnel.

*Sodium Citrate* (20 per cent. Solution).—Purified as for sodium tungstate solution.

*Ammonia Solution*, S.G. 0.88, of low copper content.

*Sodium Diethyl-dithio-carbamate*.—0.2 per cent., stabilized with 10 ml. of ammonia per 100 ml., as recommended by Clare *et al*(3). Kept in a brown bottle and renewed after three weeks.

*Iso-amyl Acetate*.—Following the recommendation of Drabkin(4), iso-amyl acetate (B. Pt. 136° C. to 140° C.) was found preferable to iso-amyl alcohol for extracting the copper carbamate colour. Lower boiling-point solvent should not be used, as it is not the iso-amyl acetate.

*Standard Copper Solution*.—0.3928 g. of copper sulphate (Analar) are weighed out and made up to 100 ml. with glass-distilled water, two or three drops of concentrated sulphuric acid (Analar) being added to prevent the formation of insoluble basic copper sulphate. From this solution 1 ml. = 1 mg. Cu) further dilutions are made up; (a) 1 ml. = 0.1 mg. Cu; (b) 1 ml. = 0.01 mg. Cu; (c) 1 ml. = 0.001 mg. Cu.

#### *Filtration Method*

Prior to weighing out, the bottles containing the samples of cream are placed in a water-bath at 30° C. to 35° C. for ten to twenty minutes to soften the fat and then shaken gently or inverted a number of times to mix thoroughly. With samples of high fat content, especially if the bottles have been filled rather full, careful stirring is often needed to obtain a homogeneous sample, but care must be taken not to churn the cream by too vigorous shaking. When several portions are required for one sample they should be all weighed out on one occasion, as subsequent warming and remixing may not give representative portions. After washing the top of the bottle and the outside of the rubber stoppers first with a swab of cotton-wool moistened with dilute acid, and then with warm glass-distilled water, the stopper is removed and 25 g. portions of the well-mixed cream are weighed out, by means of a wide-mouthed pipette, into numbered centrifuge tubes with capacities of at least 50 ml. (The size of the tubes is governed by the centrifuge available. A 50 ml. Pyrex tube about 4½ in. by 1½ in. diameter has been used, but a tube approximately 4½ in. by 1½ in. diameter, capacity about 105 ml. is preferable.)

Cream differs from butter in requiring the emulsion to be broken before the fat can be readily separated from the aqueous phase, so each sample must be churned as was done by Barnicoat(1) in making small experimental lots of butter. To facilitate churning and also to ensure extraction of copper, 2 ml. of hydrochloric acid (C.B.) should first be added (1 ml. of nitric may be used instead.)

After closing the tubes with smooth acid-washed rubber stoppers, they are chilled in ice for an hour or more to harden the fat prior to churning. A number of tubes may be placed in a power-operated mechanical shaker, but prolonged shaking after the butter has separated should be avoided. When properly churned, the sample should consist of a small compact lump of butter together with the resultant buttermilk. The tubes are cooled and, before opening, the lips and the outside of the rubber stoppers are carefully washed with glass-distilled water. The portion of the rubber stopper inside each is also rinsed, with a small amount of glass-distilled water, into the appropriate tube.

The samples are then placed in a water-bath at 45° C. to 50° C. as in the method for butter. When the fat has melted, 2.5 ml. of trichloroacetic acid (20 per cent.) is added to each tube, which is then gently shaken. To each is added 1.5 ml. of sodium tungstate solution, the tubes again being gently shaken, after which they are allowed to remain in the water-bath at 45° C. to 50° C. for half an hour. The samples are then centrifuged at about 1,000 to 1,500 r.p.m. for five to ten minutes to separate the fat, so that most of it can be drawn off by suction. In dealing with watery samples of cream or with buttermilk, more protein precipitant is required. For example, in one set of samples the protein content of a cream sample was 1.96 per cent., while the resulting buttermilk contained 2.97 per cent. protein. On this account the amount of 20 per cent. trichloroacetic acid was usually increased to 5 ml. for buttermilk, or rather less for cream samples in which the watery phase was greater than normal.

To solidify the remainder of the fat, the tubes are cooled (preferably by leaving overnight). The samples can then be filtered through wet filter papers (Whatman No. 40, 42, or 50) which have been previously washed with dilute nitric acid and glass-distilled water. Owing to the greater bulk of the protein precipitate from cream, larger filter papers (12.5 cm.) were required than in the case of butter.\*

To wash the precipitate, 2 ml. each of 5 per cent. trichloroacetic acid and 5 per cent. nitric acid are mixed together in the centrifuge tubes and the rinsings poured over the precipitate, using a stirring-rod to direct the washings round the upper portion of the paper. Three such washings are usually sufficient. The precipitate then on the filter paper is given a final washing with 2 ml. each of trichloroacetic acid (5 per cent.) and nitric acid (5 per cent.), followed by about 5 ml. of distilled water.

To each filtrate is added 5 ml. of sodium citrate solution (20 per cent.) and 1 drop of phenolphthalein indicator, followed by 0.88 ammonia drop by drop with shaking until a decided pink colour is obtained. The filtrates were then made up to the same volume, usually 50 ml., and 2 ml. of the carbamate reagent added. After adding 5 ml. of iso-amyl acetate, the tubes are placed in a water-bath at 55° C. to 65° C. for five to ten minutes prior to shaking twenty-five to thirty times. The acetate solvent usually clears more readily on standing than the alcohol.

\*If 6 in. by 1 in. Pyrex test-tubes are used to collect the filtrates, they may later, before shaking, be closed with cellophane-covered rubber stoppers. Glass-stoppered test-tubes, B.S.S. No. 770, 1938, with stopper C. 19, are a valuable refinement.

*Wet-ashing Method*

To duplicate 25 g. samples of cream contained in 105 ml. centrifuge tubes, 10 ml. of concentrated nitric acid is added. The tubes are then placed in a water-bath at 60° c. to 70° c. for ten to twenty minutes, while the contents are stirred every few minutes with L-shaped glass rods to break up the froth formed by the vigorous evolution of gas in the early stages of the digestion. As soon as possible the temperature of the water-bath is raised to near boiling-point and the samples allowed to remain therein for forty to sixty minutes with occasional brisk stirring. When gassing has almost ceased the samples are cooled in ice-water or preferably set aside overnight to allow the fat to solidify, after which the acid layer is poured or pipetted off into a 200 ml. Kjeldahl flask. (This modification of the procedure used with butter samples was adopted after discussion with Mr. W. H. Udy, who had found it an improvement on the benzoline extraction method formerly used in his laboratory by Williams(10).) A further 2 ml. of concentrated nitric acid are then added to each sample, which is replaced in the water-bath at 60° c. to 65° c., where it is vigorously stirred several times. After ten to fifteen minutes, approximately 10 ml. of hot distilled water are added to each. When the fat has separated from the aqueous layer the latter is pipetted off into the Kjeldahl flask. The fat is then washed with 15 ml. to 20 ml. of hot glass-distilled water, which is also pipetted off into the appropriate Kjeldahl flask.

The combined acid and aqueous washings are evaporated down to 5 ml. to 8 ml. and 1 ml. to 2 ml. of concentrated sulphuric acid added to each. Heating is continued until signs of charring begin to appear, when a further 2 ml. of concentrated nitric acid are added. This is repeated until the sample remains clear on strong heating, 5 ml. to 9 ml. of concentrated nitric acid usually being sufficient to complete the digestion, provided the minimum amount of fat has been transferred to the Kjeldahl flask. If too much fat is present after 6 ml. to 8 ml. of nitric acid have been used, three to five drops of 30 per cent. hydrogen peroxide are added to facilitate digestion.

When fumes of sulphuric acid begin to be evolved from the colourless liquid the heating is stopped, the flasks allowed to cool, and about 10 ml. of distilled water added to each. This is gently boiled off to ensure removal of traces of peroxide or nitric fumes. The residual sulphuric acid and crystals are poured into the extraction tubes and the flasks rinsed with a few millilitres of distilled water. To ensure the solution of material such as calcium sulphate which might adsorb traces of copper(8), 3 ml. of constant boiling hydrochloric acid are added to each flask, and after heating almost to boiling the contents are transferred to the extraction tubes and finally washed out with hot distilled water.

After the addition of 5 ml. of sodium citrate (20 per cent.), a drop of phenolphthalein indicator is added to each extraction tube and the contents made alkaline, using approximately the same amounts of ammonia for each tube. The colour is developed with carbamate reagent. The liquid is then warmed, and extracted with iso-amyl acetate, as in the filtration method.

### *Final Estimation*

With each set of samples, blanks and standards are made up. The blanks should contain all the reagents, and must be treated in the same manner as the samples—*i.e.*, in the filtration method, heated and filtered to remove tungstic acid; in wet-ashing, digested to boil off the nitric acid, &c. The standards should contain C.B. hydrochloric acid for filtration, and sulphuric acid for the wet-ashing method, together with the same amounts of sodium citrate, phenolphthalein, and ammonia as in the samples. The carbamate colour is extracted similarly with amyl acetate.

For the comparison of the colour of the unknowns with the standards a Klett bio-colorimeter with micro-cups was at first used. Another simple and convenient method is based on the use of the Lovibond comparator disk for copper, graduated from 2.5 to 50 $\mu$ g. On matching the disk with the test amyl acetate extract, the disk reading divided by 50 gives the copper figure directly as parts per million. Though not quite so accurate, this enables a series of readings to be quickly made.

The most satisfactory and reliable method of measuring the colour is by means of a photo-electric absorptiometer. A simple instrument has been constructed, similar to that described by Strafford(7). To provide a steady source of light a 6-volt secondary battery is connected to a 3-watt lamp (with adjusting rehostat). The light from this is passed through a round 100 ml. flask containing copper sulphate solution (about 1 per cent. acidified with a little sulphuric acid). This acts as a filter to eliminate certain wave-lengths (especially infra-red, as recommended by Bolton and Williams(2)) and as a lens which brings the pencil of light to a focus. Thence the light rays diverge before reaching a Weston photonic cell, which is connected to a sensitive mirror galvanometer. At the focal point is placed a holder into which is slipped a tube containing the coloured solution. By using a pipette operated by a rubber teat the acetate extracts are transferred into a selected series of uniform tubes, which are placed in turn in the holder. The galvanometer readings are quickly taken, and by plotting a graph based on the standards the results can be obtained. With this absorptiometer the method used is sensitive to 0.03 $\mu$ g of copper.

A slight turbidity of the amyl acetate solution does not interfere with the use of the Lovibond comparator disk, but may cause the Klett colorimeter readings to be less reliable. For the photo-electric instrument clear solutions are essential and are usually obtained without centrifuging if the aqueous filtrates are warmed before shaking with acetate.

### DISCUSSION

In evolving these methods a considerable amount of preliminary work was done by E. D. Andrews(6) during the investigation of methods for copper in butter, as already published. After many trials to ascertain the reason for various discrepancies which were encountered, close agreement between the methods was achieved by following the procedure described. As already mentioned, due care is needed in mixing the samples when weighing a series of portions. In the filtration method, owing to the larger proportion of protein and other milk solids contained in cream as compared with butter, increased amounts of reagents are required. Up to 2 ml. of 20 per cent. sodium tungstate solution have been tried, but 1.5 ml. of 10 per cent. is usually sufficient. Less may suffice if the protein content

of the sample is low. Variations in the amounts specified of hydrochloric or trichloroacetic acids have not been found necessary, except for butter-milk as described earlier.

Eden and Green(5) have stressed the importance of using sufficient citrate for the de-ionization of iron and the prevention of precipitation of phosphates from biological material. They also state that this process is enhanced by ensuring adequate concentration of ammonium salts in solution, and recommend the use of ammonium citrate. However, satisfactory results have been obtained with 5 ml. of 20 per cent. sodium citrate, as suggested by Tompsett(9). When an appreciably higher amount was tried, no significant difference resulted.

In the early comparisons the results obtained by the wet-ashing method were persistently rather lower than those yielded by the filtration method. To determine whether the nitric acid was removing all the copper from the fat, the latter was further extracted with 5 ml. of concentrated nitric acid at over 90° c., and again with another 2 ml. of the same reagent, and then washed with distilled water in the usual way. After allowing for blanks, only very minute amounts of copper were thus obtained (barely 0.01 p.p.m.). Eventually, low wet-ashing results were considered to be due to appreciable residues of calcium phosphate and sulphate, which would remain soluble in the concentrated sulphuric acid: but on diluting to rinse out the flask a certain amount of insoluble material often appeared. This was probably calcium phosphate and sulphate which would adsorb upon it appreciable traces of copper, as has been pointed out by Sylvester and Lampitt(8). Tests made upon calcium sulphate showed that it could readily be dissolved in strong hydrochloric acid, so the use of this was adopted to ensure the removal in soluble form of the salts left in the digestion flask.

Low copper results were also found to be sometimes due to fading of the copper carbamate colour when the excess of hydrogen peroxide had not been removed. On the other hand, high copper results have been noticed, due to ferrous iron. This may give rise to a brownish colour in the acetate extract, but does not occur so readily if the iron is in the ferric condition. In some filtration trials where low results were obtained, further copper was recovered by carefully washing the residues from the filter papers into a series of Kjeldahl flasks and applying the wet-ashing method.

### RESULTS

The copper content of samples of raw cream from various sources has only rarely been found to be below 0.10 p.p.m. Fifteen samples delivered by suppliers to different factories ranged from 0.10 to 0.14 p.p.m., with an average of 0.12 p.p.m. Eleven similar samples in the range 0.16 to 0.20 gave an average of 0.18 p.p.m., but in the case of these, as well as of others giving still higher figures, copper was probably picked up from milking-machines or coolers. Samples were procured from one factory which obtained its cream chiefly from several skimming-stations to which suppliers delivered their milk to be separated. The cream from these skimming-stations gave the following copper figures: 0.09, 0.10, 0.12, 0.13, 0.15, 0.15, 0.43 p.p.m. (It is of interest to add the iron figures for these samples, which were respectively 0.65, 0.68, 0.89, 1.05, 1.07, 1.00, 1.15 p.p.m.) The last high copper figure was due to the excessive use of a chlorine sterilizing compound which had removed a great deal of the tinning from brass pipes and fittings in that particular skimming-station.

In the following tables are given some of the results obtained by the two methods from samples of different origin. The figures in Table I show that the cream initially is of low copper content and is picking up very little copper off the plant. The higher proportion of the copper goes into the buttermilk, leaving butter of quite low copper content. This suggests that when high copper figures are obtained for butter, the cream from which it was made must have contained more copper.

TABLE I.—SAMPLES OF LOW COPPER CONTENT

	Filtration Method.		Wet-ashing Method.	
	P.p.m.	Average.	P.p.m.	Average.
(1) Cream from neutralizing-vat before pasteurizing	0.12	} 0.12	0.15	} 0.14
	0.12		0.13	
(2) The same cream after pasteurizing and cooling	0.11	} 0.11	0.13	} 0.13
	0.11		0.13	
(3) Buttermilk from the cream ..	0.14	} 0.15	0.15	} 0.15
	0.15		0.15	
(4) Butter from the cream .. ..	0.07	} 0.07	..	..
	0.07			

TABLE II.—COMPARISON OF METHODS

	Filtration Method.		Wet-ashing Method	
	P.p.m.	Average.	P.p.m.	Average.
(1) First cream from receiving-vat ..	0.24	} 0.23	0.22	} 0.22
	0.23		0.22	
(2) First cream flowing into refrigerated vat A	0.46	} 0.48	0.46	} 0.48
	0.49		0.50	
(3) Later cream flowing into refrigerated vat A	0.32	} 0.30	0.30	} 0.30
	0.28		0.30	
(4) First cream by-passed into refrigerated vat B	0.36	} 0.37	0.29	} 0.30
	0.38		0.30	
(5) First cream from vat A flowing into churn	0.16	} 0.18	0.17	} 0.17
	0.19		0.17	
(6) Later cream from vat A flowing into churn	0.18	} 0.19	0.19	} 0.19
	0.19		0.19	

In Table II the two methods gave good agreement, except in the case of sample No. 4. In the receiving-vat, neutralizing with bicarbonate of soda is often carried out, so that the tinning is gradually worn off the copper in the bottom of the vat. While the vat is standing empty, corrosion of the copper occurs and the resulting corroded material would be dissolved by the acidity of the first cream tipped into the vat so as to give rise to the higher initial copper content shown in sample 1, as compared with the lower copper content of the bulk cream revealed by samples 5 and 6, which evidently did not pick up much copper in flowing from the vat to the churn. Lack of tinning on the pasteurizing and cooling equipment was responsible for the higher figures given by samples 2 and 3. The same effect is to be seen from the results in Table III.

TABLE III.—COMPARISON OF METHODS

	Filtration Method.		Wet-ashing Method.	
	P p m.	Average.	P.p.m	Average.
(1) Sample of first gallon from pasteurizer (very watery)	0.80 0.80	} 0.80	0.84 0.84	} 0.84
(2) Sample of first 5 to 10 gallons from pasteurizer (watery)	0.75 0.79	} 0.77	0.82 0.82	} 0.82
(3) Sampled after 50 to 100 gallons had flowed from pasteurizer	0.38 0.36	} 0.37	0.36 0.37	} 0.37

In Table IV lack of tinning also accounts for the high figures in the first three samples. When procuring these samples the idea occurred to Mr. D. Hull, Dairy Instructor, to take a sample from the large brass tap fitted to the vat. Within this tap was a large cavity in which some of the first watery liquid to flow over the plant had remained undisturbed overnight. The copper content of this proved to be very high. Later on, arrangements were made to have the tap dismantled and retinned, after which a similar sample was taken, the results of which are also shown in the table. Retinning elsewhere also enabled the improved figures for No. (5) to be obtained. These results point to the value of discarding a gallon or two of liquid from the bottom of the cream-vat before beginning to pump to the churn. After standing overnight a portion of the water which has been added to the cream in various rinsing operations usually separates at the bottom of the vat, and this can readily be run off without loss of fat prior to stirring the contents of the vat and pumping the cream to the churn. High copper figures, 1 to 6 p.p.m., have repeatedly been obtained in samples of this liquid taken from different factories, *e.g.*, Table V, sample (5).

TABLE IV.—SAMPLES WITH HIGH COPPER CONTENT

	Filtration Method.		After re-tinning	
	P.p.m.	Average.	P p m	Average.
(1) Sampled while first gallon flowing into refrigerated vat (very watery)	0.38 0.38	} 0.38	..	..
(2) Sampled while first 5 to 10 gallons flowing into refrigerated vat (watery)	0.71 0.86	} 0.78	..	..
(3) Sampled while first 50 to 100 gallons flowing into refrigerated vat	0.53 0.50	} 0.52	..	..
(4) Tap sample from vat A before cream flowed (watery)	17.7 16.7	} 17.2	1.43 1.32	} 1.38
(5) Sampled while cream flowed from vat A to churn	0.54	0.54	0.21 0.20	} 0.21
(6) Butter made from No. (5) cream ..	..	0.30	..	..
(7) Sampled while cream flowed from vat B to churn .. ..	0.70 0.63	} 0.67	..	..



In Table V are given figures for samples from another factory which were analysed by both methods with very good agreement. Further samples were procured from this factory and gave the following results (p.p.m.), which are the average of duplicates by filtration. The raw cream was again high, 0.37. The first watery sample from the cold-vat tap gave 4.4. After this, successive 2-gallon portions were drawn from the tap, and samples from these gave the figures respectively 0.61, 0.42, 0.49, 0.42. A sample after fully 100 gallons had flowed gave 0.40. The buttermilk was 0.57, while the butter gave the figure of 0.12, which was lower than might have been expected.

TABLE V.—RESULTS BY DIFFERENT METHODS

	Filtration Method.		Wet-washing Method.	
	P.p.m.	Average.	P.p.m.	Average.
(1) Raw cream ex receiving-vat ..	0.38 0.38	} 0.38	0.37 0.36	} 0.37
(2) Pasteurized cream, from first gallon entering cold vat (very watery)	0.46 0.45	} 0.46	0.47 0.44	} 0.46
(3) Pasteurized cream, after 20 to 30 gallons had flowed into cold vat	0.46 0.42	} 0.44	0.41 0.40	} 0.41
(4) Pasteurized cream, after 80 to 100 gallons had flowed into cold vat	0.36 0.34	} 0.35	0.33 0.36	} 0.35
(5) First portion from cold-vat tap (watery)	6.6, 6.3 5.6, 5.5	} 6.0	6.0 6.2	} 6.1
(6) Sample taken at churn while first cream flowing in	0.35 0.38	} 0.37	0.35 0.37	} 0.36
(7) Sample taken at churn after 20 to 30 gallons had flowed	0.34 0.33	} 0.34	0.31 0.32	} 0.32
(8) Sample taken at churn after about 200 gallons had flowed	0.34 0.35	} 0.35	0.34 0.33	} 0.34

From a factory which usually had a low copper content in its butter a sample was on one occasion received with a much higher figure. On inquiring about this, the following report was received from the Dairy Instructor: "The butter in question was made from second-grade cream and can-steamings and prior to churning was stored in a copper-lined rectangular direct expansion vat, showing some bare copper." In another case where high copper figures were obtained the Dairy Instructor found on inspection that most of the tinning had disappeared from the copper tray at the top of the coolers which received the hot cream from the pasteurizers. Elsewhere there was also bare copper to be seen.

Finally, details may be given of a very striking case where the origin of the copper contamination in a certain factory puzzled experienced Instructors. Interspersed with lower copper figures, rather high results were yielded by occasional butter samples from this factory during a period of three or four years—e.g., 0.08, 0.45, 0.41, 0.22, 0.14, 0.07, 0.14, 0.13, 0.16, 0.50, 0.25, 0.18, 0.73, 0.45, 1.70 p.p.m. The Dairy Division's London Inspector reported that in a faulty box of butter made by this factory a white tallowy portion was found to have a copper content of 25 p.p.m., while the adjoining normal butter gave a figure of 0.3 p.p.m. Another box examined at the same time had 0.6 p.p.m. of copper. Latterly when higher figures became more noticeable, senior members of the Dairy Division's staff expressed surprise at the possibility of serious copper contamination in that factory as nearly the whole of its plant was made of stainless steel, and a check-up on the cream supply was suggested to find if copper was coming from farm utensils. Before doing this, samples of cream from various parts of the plant were procured and yielded the results given

in Table VI. While these samples were being analysed, another sample of butter gave the extraordinarily high result of 1.70 p.p.m. Further samples were then procured by an Instructor who had previously searched the plant for bare copper. The results are given in Table VII. The three water samples had been lying in the respective gun-metal pumps for over twenty-four hours. Prior to taking the cream samples, water was flushed over the plant for about twenty minutes. This served to remove thoroughly the copper contamination which was originating apparently from the gun-metal pumps of the vacreator. Since this practice of a thorough preliminary rinse with water has been maintained, further samples of butter have yielded much more satisfactory copper figures, as follows: 0.10, 0.11, 0.11, 0.11, 0.08, 0.08, 0.09, 0.08, 0.09, 0.13, 0.10, 0.13 p.p.m.

TABLE VI.—STAINLESS-STEEL EQUIPMENT AND COPPER CONTAMINATION

	Filtration Method		Wet-ashing Method.	
	P.p.m.	Average	P.p.m.	Average.
(1) Raw cream from receiving-vat ..	0.12 0.13	0.13	0.13 0.11	0.12
(2) First cream into holding-vats (very watery)	0.59 0.56		0.62 0.62	
(3) Cream into holding vats after ten minute run	0.22 0.25	0.24	0.25 0.23	0.24
(4) Start of cream through pipe into churns	0.23 0.23		0.19 0.20	
(5) Cream into churns after agitation ..	0.23 0.21	0.22	0.21 0.25	0.23
(6) Cream into second round of churns ..	0.21 0.21		0.20 0.18	

TABLE VII.—SOURCES OF COPPER CONTAMINATION

	Average (p.p.m.)	Previous Results (p.p.m.).
(1) Water from cream-pump to vacreator (about 25 ml., opalescent)	9.0	..
(2) Water from cream-pump from vacreator (about 140 ml., milky)	15.0	..
(3) Water from cream-pump from water-cooler (about 30 ml., almost clear)	0.53	..
(4) Raw cream in receiving-vat .. .. .	0.12	0.12
(5) First cream through vacreator and coolers (very watery) ..	0.15	0.62
(6) Cream through vacreator and coolers near end of run ..	0.14	0.24
(7) Butter made from above cream .. .. .	0.08	..

## CONCLUSION

Many careful experiments have been carried out in order to improve the techniques of both the filtration and the wet-ashing methods. They now give results which agree closely. The results given in the tables demonstrate how such methods can be applied to track down unsuspected faults in the butter-factory machinery. The filtration method has the advantages of avoiding objectionable acid fumes, risks of contamination are reduced, and the results are more simply obtained. By using this simplified method, Dairy Division Instructors can be readily provided with

information to enable them to attempt to eliminate at the source potential causes of spoilage in butter due to copper contamination, instead of having to wait for defects in the stored products to be complained of by consumers.

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## EQUIPMENT FOR THE SULPHITING OF APPLE SLICES

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### Summary

A description is given of a machine designed by the Chemical Engineering Section of the Dominion Laboratory for dipping apple slices into sulphite solution in commercial quantities. Reference is also made to two earlier devices. All three machines are capable of dealing with one thousand cases of apples per day and have been used in the preparation of apples for dehydration.

WHILE for many years the customary method of protecting apples from oxidative effects during drying has been by exposing the peeled or sliced apple to the fumes of burning sulphur, recent work has shown that a more uniform and generally improved product could be obtained by dipping the apple slices into a solution of sodium metabisulphite. With this method close control can be obtained of the amount of sulphur dioxide added to the apple and, if desired, it is possible to add greater amounts than is possible with sulphuring in the normal way. As the writer was concerned only with the engineering side of the project, it is not proposed to discuss the chemistry of the addition of sulphite or sulphur dioxide to apples. Work on this aspect was carried out at the Plant Chemistry Laboratory, Palmerston North.

When the proposal was first put forward it was believed that a dipping time of some six to ten minutes was required, and therefore a batch type of dipping mechanism was installed. With this considerable variation could be obtained in the time of dip. This equipment is illustrated in Fig. 1. The unit was installed at Hastings and has operated for several seasons. It has the disadvantage that it requires an attendant to operate the mechanism, but control is simple and very little trouble has been experienced. To avoid contamination, all parts coming into contact with the apple or the solution were made of wood, rubber, or other material not attacked by the apple-juice.

When a factory was erected later at Motueka it had been determined that a much shorter dipping time was satisfactory, and a continuous dipping unit was installed. This is illustrated in Fig. 2. This consisted of a wooden vat 17 ft. long by 2 ft. wide over which ran a slatted conveyor belt. The apples were delivered into one end of this vat, then carried down into and through the sulphite solution in the vat by means of the wooden slats attached to the rubber belt. At the end of the vat the slices were carried up a sloping extension and fell over on to the end of a tray conveyor. The belt was driven by a variable-speed reduction gear, the normal speed used giving a dipping time of about half a minute. In 1945 the wooden vat was replaced by stainless steel and a sump was formed at one end so that pips, &c., could fall clear of the slices and be eliminated from the product.

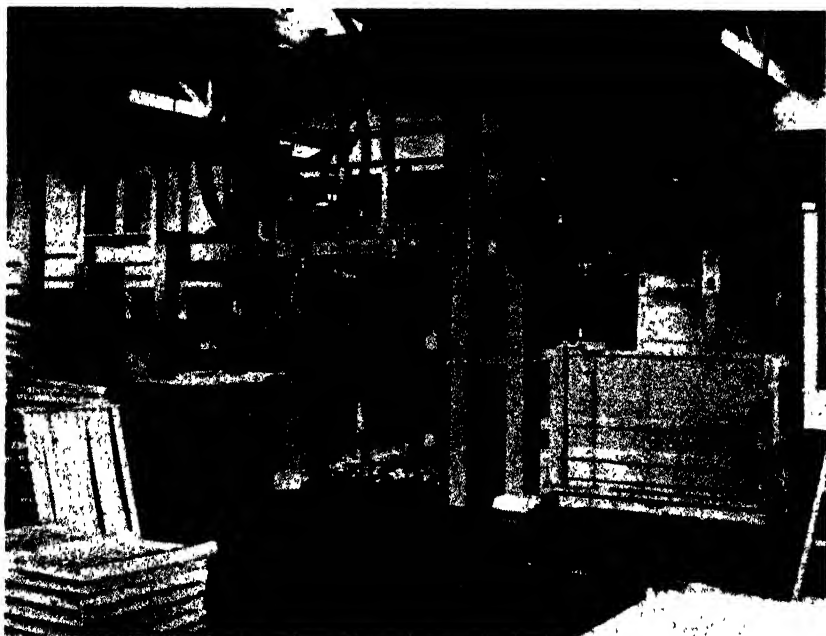


FIG. 1.

While having satisfactory capacity, certain defects were evident, particularly the difficulty of satisfactorily mixing the solution in the long tank. The unit also required a great deal of floor space, and, as will be seen from the photograph, a considerable quantity of apple was carried back on the belt to travel through the bath a number of times before delivery to the trays.

In view of these and other defects, and since storage tests had shown that an almost instantaneous dip into the solution was satisfactory, it was decided to build a much more compact machine and one in which the defects in the two previous machines were eliminated. A new machine was designed, a photograph of which is shown in Fig. 3. Figure 4 is a diagram showing the dipping portion of the bath.

Essentially the machine consists of a stainless-steel tank 4 ft. long by 2 ft. wide by 2 ft. 6 in. deep. Into this projects a stainless-steel drum 3 ft. in diameter driven by motor and reduction gear at a speed of approximately two revolutions per minute. This drum is set so that it dips into the solution

held in the tank at a controlled depth. From the drum twelve paddles project through slots. These paddles are mounted on a shaft held a short distance below the centre of rotation of the drum so that as the drum rotates it causes the paddle to turn with it, and these, rotating about a different centre, are ejected and withdrawn into the drum during each revolution. As the paddles withdraw through the slots in the drum, any apple slices adhering are scraped off by the drum. On further rotation the drum itself is scraped clean by a rubber scraper shown in the sketch. Inside the tank a curved perforated division is placed just so as to clear the paddles. This is connected to the discharge chute. A drain cock and float valve for the control of the level are also provided.

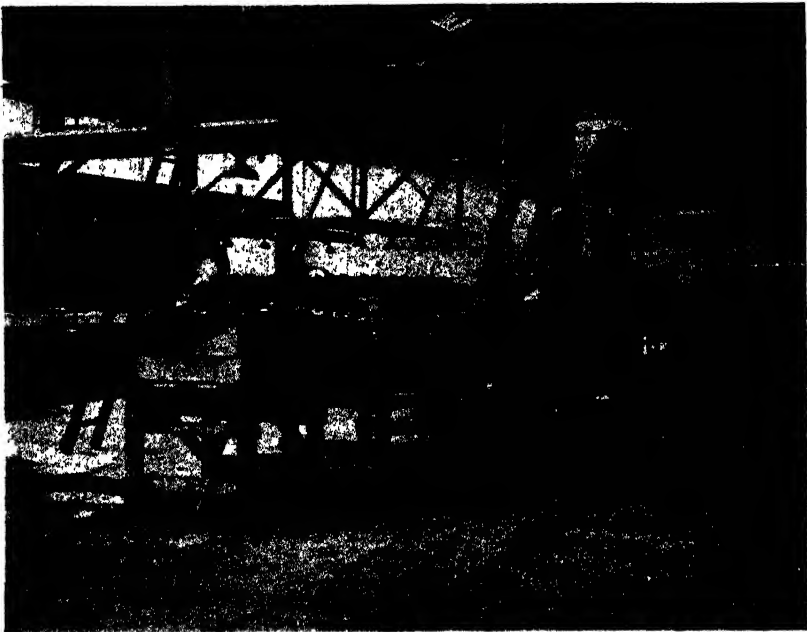


FIG. 2.

The apples are discharged from a conveyor into the free space in the tank beside the float chamber. As the apple slices float on the surface they are carried by the flow of the solution to a point where they are collected by the paddles. They are then carried down under the solution and pushed up the perforated division until they fall over the end on to the discharge chute.

During the passage up the division, draining can take place so that solution loss is minimized. The wetted slices are scraped off the paddles and the drum by the means described so that all fall down the discharge chute on to the spreading belt.

In Fig. 3 it can be seen that the dipper delivers on to a spreader belt driven by the chain sprocket. To obtain even spreading, the belt passes below a "diamond spreader." This has been modified from the original American design by providing screw adjustments for all four corners of the diamond and increasing the width of the diamond to 6 in. Running the spreader belt at approximately half the speed of the tray conveyor it is possible to obtain a uniform ribbon of material on the belt and the desired

weight of apple slices on a tray. Occasional whole apples do not jam under the diamond, but have to be picked off the trays before they pass into the tunnel. With two operators, one loading the empty trays on to the conveyor and the other loading the filled trays on to the trolleys, and with the tray conveyor running at 30 ft. per minute, the machine will load one hundred



FIG. 3.

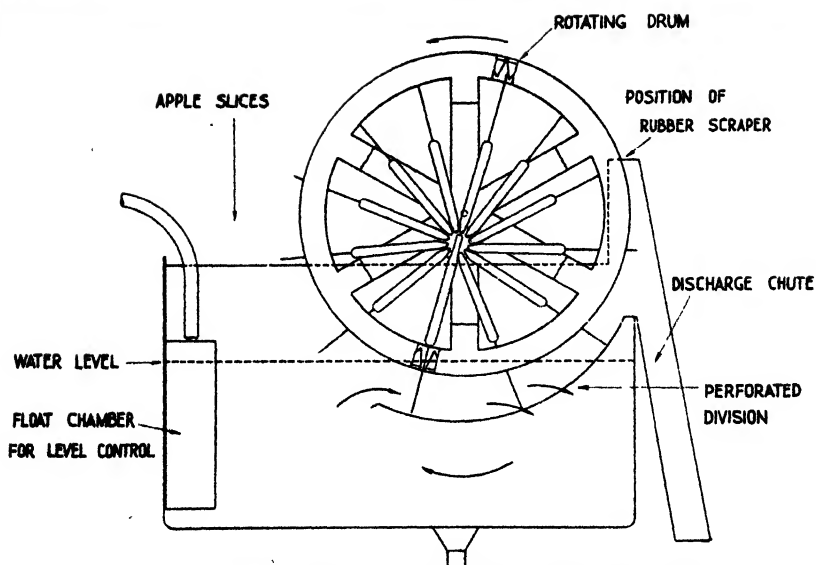


FIG. 4.—Diagram showing dipping portion of sulphiting bath.

trays in ten minutes. Allowing for the time required to bring a fresh trolley into position and occasional stoppages, it is possible to load four trolleys per hour or, at a tray loading of 9 lb. per tray (approximately  $1\frac{1}{2}$  lb. per square foot), the machine will handle 3,600 lb. trimmed and sliced apple per hour.

This is sufficient capacity to keep one dehydration tunnel in operation for sixteen hours with the spreader operating a single shift. Complete working drawings of this unit are available from the Dominion Laboratory on request.

#### ACKNOWLEDGMENTS

The writer wishes to express his appreciation of the co-operation of the Internal Marketing Division, by whom the Motueka factory is operated. Thanks are expressed to Mr. A. P. Druce, who carried out the design of the equipment, and to the Director, Dominion Laboratory, for permission to publish this paper.

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NUMBER 5

## SILAGE STUDIES

### IV. A COMPARISON OF LOSSES IN PASTURE SILAGE MADE IN PITS AND IN STACKS UNDER VARIOUS CONDITIONS

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(Continued from Vol. 24, No. 3A, page 130A)

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#### Summary

(1) Losses in chemical constituent and in digestible nutrients of pasture silage were determined over a four-year period in 25-ton concrete pits and 50-ton stacks. The results are therefore derived from over 800 tons of ensiled material.

(2) The average percentage losses of dry matter (D.M.), crude protein (C.P.), and organic matter (O.M.) are—

	D.M.	C.P.	O.M.
Pits .. .. .	34	35	46
Stacks .. .. .	42	41	42

(3) The apparent digestibility values as determined by feeding trials (expressed as percentages) are—

	D.M.	C.P.	O.M.
Ensiled herbage .. .. .	74	74	76
Pit silage .. .. .	64	49	66
Stack silage .. .. .	62	36	64

(4) The average percentage losses of total digestible constituents are—

	D.M.	C.P.	O.M.
Pits .. .. .	42	61	47
Stacks .. .. .	51	73	51

(5) The average losses of dry matter as inedible material were 2.6 per cent. for pit silage and 10.2 per cent. for stack silage.

(6) Wastage on the circumference of stacks was highest in the year when the percentage of dry matter in the material ensiled was greatest (over 27 per cent.).

(7) The average loss of dry matter in exudates from pits was 6.1 per cent., as compared with 3.2 per cent. in stacks, and was greatest in those seasons when the percentage of dry matter in the material ensiled was lowest (below 20 per cent.).

(8) The addition of mineral acids markedly lowered the losses in dry matter, but such silages were less palatable, even after neutralization by ground limestone.

(9) Adequate consolidation of the top layers of stacks by means of an earth cap reduced losses appreciably, but protection against rain showed no beneficial effect.

(10) The losses recorded are believed to be a very conservative estimate of the average losses occurring in normal farming practice in New Zealand.



## INTRODUCTION

In previous papers from this Station results have been given of the losses occurring in experimental 2-ton silos(1) and of the chemical composition and digestibilities of pasture silage from full-size stacks and pits(2, 3, 4). The losses recorded were relatively high, and since this might be associated with the size of the silos used, it was considered necessary to repeat the work under conditions which approximated as closely as possible to the best farming practice. Such an investigation would not only give a comparison between small- and large-scale ensilage procedures, but would fix a reliable mean-loss figure for farm-made silage.



FIG. 1.—View of concrete tanks for collection of exudates from pits and stacks taken during construction. Pipe from bottom of pit on left allows direct measurement into the tank, from which a discharge pipe runs to connect up with the main waste seen in bottom of trench on the right. In the background can be seen a concrete stack base under construction. Collection tanks for these are at a higher level than for the pits. This allows a good fall for the total discharge pipe from the series of collection tanks.

The programme of work reported in this paper commenced in 1941, when two 50-ton stacks and two 25-ton earth pits were investigated. In 1942 two concrete stack bases to hold 50 tons each and two 25-ton concrete pits were built, all of them fitted with drains and sumps for the quantitative collection of exudates (Fig. 1). In all cases losses were determined by

weighing and sampling each load of pasture herbage before ensiling and each load of silage as it was removed. This has involved the handling during the past four years of over 800 tons of herbage and 500 tons of silage, but the method gives so much greater certainty than any of the sampling methods which have been tried elsewhere that the extra work involved was fully justified.

The data obtained from the four years' measurements are reasonably representative of the average climatic conditions experienced at Palmerston North. The seasons varied from hot and dry to cold and wet, but the average would not be abnormal. On the other hand, the quality of the herbage ensiled and the care given to the ensiling process itself was probably greater than on the average dairy-farm.

Accordingly, it is reasonable to presume that the data presented below represent a fair average of the losses being experienced under the best of North Island farm conditions of pasture growth and labour and show a conservative figure for the losses in general practice throughout the country.

Apart from the main project of normal stack and pit nutrient losses, measurements were made on stacks and pits subjected to variations in compaction, protection against rain, and also with the addition of varying strengths of acid solutions. This latter was done largely to gain experience in the use of acid treatment of large masses of ensiled herbage, but the results obtained, although of a preliminary nature, give some idea of the possibilities attending the adoption of such a practice in this country.

#### EXPERIMENTAL TECHNIQUE

The Massey College dairy pastures used for silage were closed from grazing in early October each year and cut in mid-November. Before closing, the paddocks were grazed down with cattle and sheep and harrowed to spread droppings. No nitrogenous fertilizers were used, but the pastures had an annual top-dressing of superphosphate and were limed periodically.

When ensiling commenced, a tractor mower was operated each morning from about 7.30–9.0 a.m., sufficient herbage being cut for each day's work. The herbage was raked into windrows and forked into a lorry and trailer in amounts limited by the small weighbridge used. The net weight of each load varied from 15–20 cwt. Each load was weighed separately and then immediately sampled for chemical analysis and botanical composition. A representative sample of approximately 50 lb. of mixed herbage was taken from the load, mixed thoroughly by hand, and two sub-samples each of 1,000 g. were taken for the two determinations. These sub-samples were immediately sent to the laboratory, the average time elapsing between weighing and placing the samples in gas drying-ovens being approximately half an hour. Dry-matter determinations were made after the samples had been dried at 80–90° c. for approximately twenty-four hours. The dried samples were then ground in a standard laboratory mill. Chemical analyses followed A.O.A.C. methods, but, due to staff shortages, the crude fractions only were determined. Botanical analyses were made by actual dissection of samples of 500–1,000 g. The separated constituent species were oven-dried and the percentage compositions determined on the dry weight basis.

The building of stacks and the filling of pits was carried out by the Station's farm staff, all of whom had had previous experience of silage work. Every endeavour was made to produce good silage, in so far as even stacking and filling, good consolidation, and trimming of the edges are of value.

In order to obtain an even distribution of the herbage, the loads were alternated between duplicate silos and stacks every six loads.

The filling of the pits and their over-silos took three to four days, and the stacks four to five days. Sufficient material was ensiled each day to allow for heating and settling of the mass for the next day's work.

After completion of building or filling, each stack or pit was covered by a layer of sacks, and a layer of soil approximately 24 in. thick in the middle and 18 in. on the edges. The soil layers were maintained in position by wire netting and sack aprons round the edges of the stacks, thus enabling an even distribution of weight right to the edges and preventing loss of soil by washing, &c.

Temperatures during ensiling of either pits or stacks were not recorded, except on one occasion when concern was felt about an apparent abnormal heating in one stack, a check of points 2 ft. in from the sides showing



FIG. 2.—General view of experimental stacks and pits just after completion of ensiling in the 1942-43 season. Treatments from left to right are: (a) normal stack, (b) normal stack roofed against rain, (c) normal pit roofed, and (d) normal pit.

temperatures of 125° F. The pits appeared to generate less heat than the stacks, although both developed what were considered normal temperatures.

The exudates from each silo and stack were measured daily by means of a calibrated rod in the tanks, and samples taken directly from the inlet pipe for chemical determinations.

The weighing, feeding, and sampling of the finished silages were carried out some eight months after ensiling. As each silo was opened up, the material was cut out in sections, and silage which appeared inedible was separated, weighed, and sampled separately. The inedible material was offered to the dairy herd with the edible silage and a daily check made by observing the actual feeding of the cows. Suitable adjustments were made, when necessary, in the daily classification of wastage and edible

silage. No doubt, much of the material classified as wastage would be eaten by animals on a lower plane of nutrition. The aim of silage-making, or any other form of fodder conservation, is, however, to produce fodder which is palatable at any plane of nutrition.

Digestibility trials of both the materials ensiled and the resultant silages were carried out in 1942, 1943, and 1945, using two full-grown Romney wethers for a feeding period of fourteen days and a pre-feeding period of five days on each different material. The technique adopted was as described previously for herbage and silage feeding trials at this Station(6, 4). The feeding-trial periods coincided with the harvest and the feeding-out periods of the silage crops. The feeding of the herbage started before and finished after harvest, so that the mid-point of the trial coincided with harvest, whilst the silages fed to the sheep were obtained from the amounts weighed out from the edible parts of each stack and pit.

The technique followed in adding the acid solutions to the pits in 1943 and 1944 was as follows: a bulk supply of sulphuric-acid solution of the required concentration was made up in a wooden butt holding approximately 400 gallons and mounted, at the side of the pit, on a stand 10 ft. above ground-level. A rubber hose fitted with a tap, led from the butt to the pit. The tank was calibrated in 2-gallon steps, the amount applied being controlled by inspection. All the solutions were applied at the rate of  $12\frac{1}{2}$  gallons to the ton of fresh herbage by spraying the material as it was put into the pit and spread and trampled. This volume of liquid per ton appeared to be the minimum amount that could be applied to obtain a thorough wetting of the herbage. The clothing of the operators suffered considerable damage, even though rubber boots were in use. Precautions had also to be taken to minimize danger from accidental acid burns.

## RESULTS

The measurements made have been summarized into the series of tables. (Complete details of all the measurements and feeding trials are not presented, but will be made available if required.) Tables I and II show the yield per acre and botanical compositions of the materials ensiled. Table IV gives the chemical composition, and Table VI the digestibilities of the various constituents. Tables III and VII show the compositions and losses in the silages made in the normal 50-ton stacks and the normal 25-ton pits. Tables VIII and IX set out, for ease of direct comparison, the averages of the losses in both total and digestible constituents. Tables X and XI show the different losses recorded when stacks and pits were subjected to different consolidating and coverages, while Table XII shows the different losses when solutions of sulphuric acid, in varying strengths, were added to the ensiled herbage.

The yields per acre as shown in Table I were determined by taking the total weights of the crop (green weight) as measured into the silos reduced to a per-acre basis. From a comparison with previous recorded yields(1, 7) it will be seen that the silage crops throughout were of good average quality and yield, except in two of the paddocks in 1941, which had a slightly higher content of lower producing species of grasses and a lower total yield.

Table II shows the botanical compositions of the herbage actually ensiled in the various treatments each year. These data are presented in detail because the herbage ensiled could not be drawn from one complete paddock of one composition, and also because the stacks and pits were

filled in sequence. However, the composition of the materials ensiled in the various treatments in each year was sufficiently similar to enable valid comparisons between treatments. Table III sets out the weights of the herbage ensiled, the weights of the silages recovered, and the exudates. These represent the total weights and are subject only to the dry-matter-sampling error. This latter, however, should not be large because of the great number of determinations made over the seasons by sampling every load in and out of the stacks. The apportionment of the percentage losses of dry matter has been made by direct calculation of the dry matter recovered in the edible and inedible silage and in the exudates. The differences between the sum of these and the total dry matter ensiled are expressed as "invisible" losses—i.e., losses due to the respiration and fermentation processes. Also included will be any losses of actual silage around the edges of the stacks or an incomplete collection of the exudates, but there was no reason to believe that these were of any significance.

The losses in the form of total exudates were greater in the pits than in the stacks in all cases, the amounts varying directly with the moisture content of the ensiled herbage. The losses in the form of wastage were greater in all cases in the stacks than in the pits, but no relationship can be seen between such losses and the state of the material, except perhaps in the case of the 1942-43 stack. In this year, when the dry-matter content was high (27.6 per cent.), it would appear that consolidation was not as good as in the other years, and losses as wastage may have been higher for this reason. It is not reflected in the same material ensiled in pits, however, but this is explicable by the anaerobic conditions in the concrete silo. Invisible losses of total dry matter show no definite differences as between stacks and pits, but total dry-matter losses were definitely lower in both types in the 1942-43 season than in the others. In this year the herbage was drier than in other years and probably gave better conditions for fermentation processes, although this was counter-balanced to some extent by the greater amount of inedible wastage in the stack for that year and the lowered digestibility values.

The chemical compositions as shown in Table IV were determined on bulked amounts of aliquot weights of the dried sub-samples for each load. The general composition is similar to those previously recorded at this Station(1), and by Watson(5) and others overseas. Not a great deal can be obtained from the study of such analyses in themselves, and especially so in this case, since only the crude fractions were determined. There is no great difference between the chemical composition of the silages in pits and those in stacks, but the differences are shown in truer perspective after examination of the differences in digestibility set out in Table VI, and combining all the data into one comparative examination of the overall losses of total digestible nutrients (Table VIII).

Comparing the digestibilities of the ensiled materials, it can be seen that there are no great differences between one year and another, and that there is good agreement with other samples of herbage previously examined(6, 1). The general high levels of digestibility of all the constituents of high-producing New Zealand pastures have been discussed previously(6). Considering the digestibilities of the silages, however, it is seen that there was a considerable drop in the protein and more-soluble carbohydrate constituents. The decreases were generally greater in the stacks than in the pits, and it is reasonable to assume that not only did leaching of the more soluble nutrients take place, but also a decrease occurred in protein digestibility as a result of the greater heating in the stacks.

There was a variation from year to year in both stacks and pits, but only in the 1942-43 season did the drop in the digestibilities of the pit silage assume the large proportions it does in the stack silage.

It is of interest to compare the mean figures obtained for all the digestibilities with other recorded data. In the case of the pit silages the average figures for the four seasons are very close to those previously recorded at this Station for silages made with similar materials and in similar pits(7) and also those made in smaller (2-ton) concrete pits(1). Digestibilities are lower than those quoted by Watson as typical figures for silages made in towers and lined pits, but not markedly lower than those given for pit silages, although the drop in digestibility in this case is greater, due to the higher digestibility of the ensiled material(5). The digestibility figures for the stack silages compare reasonably well with the similar range of silages made in England(5) and also with stack silages of similar composition in America(8). The only figures available for comparison within New Zealand are those of a wide range of samples collected on the survey referred to(2). For these, pepsin solubility of nitrogen was used as an index of digestibility, and if this can be accepted, the digestibilities of the stack silages measured here compare very favourably with the best that were examined in the field then.

Tables X and XI, showing the losses recorded for stacks and pits with different coverings, call for little comment. The greater losses due to the lack of earth consolidation are definite for both years. The results are shown visually in Figs. 3-5. Greater losses of digestibility would undoubtedly have been recorded for the large amount of darker-looking silage on the top of the unweighted stack, and the total loss of digestible nutrients would be even greater than revealed in the tables. It seems reasonable to conclude(5) that the required minimum weight of consolidating earth cap will equal the weight of the mass of herbage affected by the lack of consolidation, shown visually in this case by its darker appearance. Roofing against the weather resulted in an increase of total dry-matter losses in both years on the stacks and in one year in the pits, with a reduction in one year in these latter. The great reduction in losses in roofed pits previously recorded here was not repeated in the larger structures and is probably associated with the size of the silos, as well as the fact that the concrete silos were built with a projecting lip. This prevented the ingress of outside surface drainage, which may have happened in the earth pits and also in the smaller experimental group previously measured. Also, the unroofed pits and stacks, by reason of exposure to rain, had the benefit of a heavier earth cap throughout than did the roofed silos. The effect was noticed more in the stacks than the pits, as it would seem that the weight of the consolidating caps on the former, even with wet soil, was insufficient for thorough compaction of the mass.

Table XII sets out the reduction in losses when solutions of sulphuric acid were added to the ensiled herbage. In every case the reduction of losses and increase in total acidities were considerable and approximately in direct proportion to the strength of the acid solutions used. The data collected do not, however, allow of any full discussion of the effects on the silages themselves, and the figures obtained are presented as a simple statement of the gross results. The acid silages proved very unpalatable to dairy cattle, even after neutralizing with appropriate quantities of lime and soda mixtures. The refusal of sheep to eat silage made with added acids prevented the carrying-out of digestion trials on this class of silage material. It should be remembered, in considering direct acidification of silage as a means of reducing losses, that costs of acid are much higher in

New Zealand than in other countries. This, together with the difficulty of applying acids and of neutralizing the resultant silages, would no doubt prevent its adoption in practice, no matter how effective it were in reducing losses. Similar views are held by several American workers(12).

No figures have been quoted for pH levels. pH determinations were recorded for both the exudates and final silages, but have not been quoted because considerable variations in the values obtained and a number of anomalous recordings. Without more detailed measurements of the acid constituents themselves, together with parallel bacteriological work, the pH figure alone is of limited value.

#### DISCUSSION

The measurements made over the whole programme of work reported show losses ranging from 50 per cent. down to 14 per cent. of the total dry matter ensiled, with similar variations in digestibility losses depending on



FIG. 3.—View of unweighted silage stack. Instead of adding soil to compact the mass, some farmers prefer to cone the stack, both to turn the water and also to give extra consolidation in the centre. Photo should be compared with treatment (a) in Fig. 2 to see difference in stacking method. Much greater nutrient losses were recorded for this method of stack-building than for the normal weighted silage stack.

the constituent and the process used. The work has supported the results obtained in Europe and America, although there appears to be a greater general net loss under New Zealand conditions, due to the high digestibilities recorded here for silage crops of similar maturity, yield, and chemical composition.

Most of the overseas reports on silage-making, in their discussion of New Zealand results, assume and emphasize a high level of silage quality in this country. This is no doubt due to the fact that there has been no actual measurement of losses made here previously, and the statements issued to date have been based largely on observation with a limited amount of chemical analyses of the cured silages.

It is a matter of considerable importance to pasture and animal production in New Zealand that the normal losses as recorded in the work reported here, and as previously suggested(1, 2), are as high as, if not higher than, in other grassland countries. Hamilton, in a survey of dairy production in New Zealand(9), established a definite correlation between the amount of hay and silage conserved and the butterfat production for the following season, and advocates, as do all grassland workers in this country, a much greater emphasis on the provision of increased quantities of supplementary feed for autumn and winter shortages.

The loss figures recorded, as well as the field-work previously referred to, suggest that at least as great emphasis should be put on the necessity to use every care in the making of pasture silage. A very general opinion held by many New Zealand farmers is that silage-making calls for no great care and that anything will do in the way of material for ensiling. Although



FIG. 4.—Profile of unweighted silage stack, with tapes attached to define limits of visually different grades of silage. The area marked "A" was inedible wastage; the silage in area marked "B" was edible, but very burnt and of low protein digestibility; the silage in area "C" was of a light green-brown colour, edible, and of higher digestibility than B.

we would be the first to admit that our efforts to make silage of good quality and with low losses have not been successful throughout, it is felt that the results secured give some useful indications and fully substantiate the recommendations of overseas workers. The moisture content of the material ensiled is a most potent factor in silage losses and quality. When the herbage is long and dry, consolidation is poor and digestibility is lowered by excessive heat; when too wet, the losses by leaching of the more soluble constituents gives a similar net result. English and American workers(11, 12) agree on the desirable moisture content as being 70 per cent. In those countries, however, chopping of the material at ensiling is common and compaction easier. From our figures for unchopped materials



it would appear that 75-77 per cent. moisture is the most suitable figure to aim at for the normal run of unchopped New Zealand pasture silage crops. The advice usually given by agricultural authorities(10) in this country, that it does not matter in what weather the ensiling is carried out, has not been substantiated, although it is realized that it is very difficult indeed to adjust normal ensiling and haymaking operations to the vagaries of the weather in the high-rainfall areas of New Zealand. If the crop to be ensiled is of a rank and stemmy nature, a small amount of surface moisture may be desirable and may make compaction easier, but if it is of the leafy nature (which is the most desirable), then surface moisture should be low, to avoid excessive losses through drainage.

The superiority of the silo over the stack method has again been demonstrated by the trials reported here, but as the use of either depends on many other factors on the farm, no hard-and-fast rule can, in practice

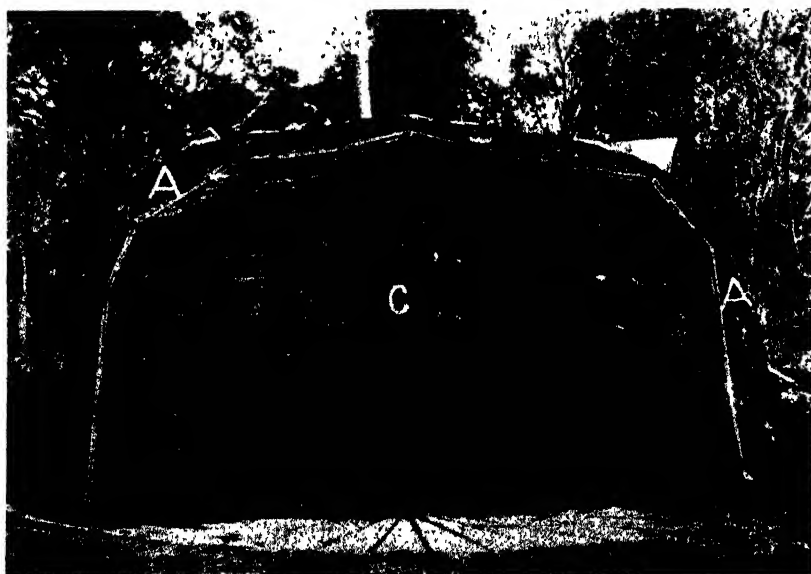


FIG. 5.—Profile of weighted stack (after removal of earth consolidating-cap). Stack face has been taped to define the limits of visually different grades of silage, as for the uncovered stack in Fig. 4. The smaller amount of inedible wastage and the elimination of the area of very low digestibility silage ("B") makes the use of earth consolidation of considerable value. Note the concrete stack base for collection of exudates. The base slopes into the centre, from which a pipe leads to the collecting-tank behind and below the stack.

be stated. Finally, although the use of acid solutions reduces losses considerably, the experience gained, together with a consideration of costs makes its practical application unlikely.

It is intended that future work should cover a detailed examination of the bacterial populations normally present in silage and measurement of their metabolic processes. It is hoped that such a study may lead to a clearer understanding of the processes involved in ensiling and possibly suggest means of reducing the present high losses associated with this method of fodder conservation.

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TABLE I.—YIELDS PER ACRE (GREEN WEIGHT) OF HERBAGE ENSILED, AND BOTANICAL COMPOSITIONS (DRY WEIGHT) OF PASTURES USED FOR THE TRIALS IN EACH YEAR

Season.	Paddock No.	Yield per Acre (Tons of Green Material).	Botanical Composition (Dry-matter Basis), per Cent.					
			<i>Lolium perenne</i> .	<i>Dactylis glomerata</i> .	<i>Poa trivialis</i> .	Other Grasses.	<i>Trifolium repens</i> .	Other Species.
1941-42 ..	5	5.6	32	18	23	2	5	Tr.
	8	5.5	49	12	20	16	2	1
	9	8.1	64	6	15	9	4	2
	21	6.8	54	21	..	15	10	..
1942-43 ..	5	8.3	49	26	16	4	4	1
	8	6.9	18	50	16	3	10	3
	9	7.4	55	12	20	1	9	1
	15	7.9	23	53	17	1	6	..
	20	7.5	69	21	1	2	6	..
1943-44 ..	3	9.4	67	10	15	2	4	2
	5	8.6	59	11	16	5	8	Tr.
	8	9.0	40	27	20	6	7	1
	9	9.4	60	6	16	6	10	2
1944-45 .	5	9.8	77	6	8	5	4	.
	8	9.6	76	6	4	8	5	1
	9	9.5	68	17	4	6	4	1

"Other Grasses" include *Holcus lanatus*, *Bromus mollis*, *Bromus catharticus* *Agrostis* spp., *Poa pratensis*, *Poa annua*, and *Anthriscanthum odoratum*.

"Other Species" include *Rumex* spp., *Ranunculus repens*, *Taraxacum officinale*, and *Plantago lanceolatum*.

TABLE II.—BOTANICAL COMPOSITIONS (DRY-WEIGHT BASIS) OF HERBAGE ENSILED IN THE PITS AND STACKS FOR YEARS SHOWN  
(Figures shown are percentages)

Season.	Type of Ensilage Structure and Treatment.	<i>Lolium perenne.</i>	<i>Deschampsia glomerata.</i>	<i>Poa trivialis.</i>	Other Grasses.	<i>Trifolium repens.</i>	Other Species.
1941-42 ..	Normal pit .. ..	42	25	17	12	4	..
	Covered pit .. ..	40	21	17	18	3	..
	Normal stack .. ..	42	30	14	3	7	2
	Covered stack .. ..	39	35	11	7	6	1
1942-43 ..	Normal pit .. ..	57	7	17	6	11	..
	Covered pit .. ..	60	11	14	6	9	..
	Normal stack .. ..	52	19	14	9	6	..
	Covered stack .. ..	54	15	19	5	6	..
1943-44 ..	Normal pit .. ..	72	13	3	7	5	..
	Acid pit .. ..	68	13	4	9	5	1
	Normal stack .. ..	74	6	6	11	3	..
	Unweighted stack .. ..	72	9	8	7	4	..
	Small silos .. ..	66	5	15	5	9	..
1944-45 ..	Normal pit .. ..	72	14	4	4	6	..
	Acid pit .. ..	72	10	6	6	5	1
	Normal stack .. ..	74	6	11	5	3	1
	Unweighted stack .. ..	72	9	12	2	5	..
	Small silos .. ..	71	17	3	..	9	..

TABLE III.—TOTAL WEIGHTS OF MATERIAL ENSILED IN STACKS AND PITS AND THE WEIGHTS RECOVERED OF RESULTANT SILAGES  
(All weights given in pounds)

Item.	Stacks.				Pits.			
	1941-42.	1942-43.	1943-44.	1944-45.	1941-42.	1942-43.	1943-44.	1944-45.
Ensiled herbage—								
Green herbage ..	101,150	100,122	99,805	99,940	49,345	66,016	60,946	70,727
Dry matter ..	23,786	27,673	20,737	18,492	10,010	17,808	13,430	11,799
Silage recovered—								
Wet weight—								
Edible ..	59,238	60,227	55,332	50,573	35,146	57,915	43,903	41,158
Inedible ..	9,087	10,093	6,580	6,435	122	2,035	2,477	2,936
Total ..	68,325	70,320	61,912	57,008	35,268	59,950	46,380	44,094
Dry-matter weight—								
Edible ..	13,908	18,157	12,237	9,484	6,662	13,298	8,000	7,258
Inedible ..	1,994	3,697	2,143	1,608	43	499	421	490
Total ..	15,902	21,854	14,380	11,092	6,705	13,797	8,421	7,748
Exudate (weight)—								
Wet ..	*	2,803	14,000	29,350	*	5,738	16,179	36,350
Dry ..	*	190	700	1,027	*	287	808	1,272
Losses as percentages of ensiled material (dry-matter basis)—								
In inedible silage ..	8.4	13.4	10.3	8.7	0.4	2.8	3.1	4.1
In exudate ..	} 33.1 {	0.7	3.4	5.5	} 33.0 {	1.6	6.0	10.8
Invisible ..		20.3	27.3	34.5		20.9	31.3	23.6
Total ..	41.5	34.4	41.0	48.7	33.4	25.3	40.4	38.5

\* Not measured.

TABLE IV.—CHEMICAL COMPOSITION OF MATERIALS ENSILED IN STACKS AND PITS AND OF THE RESULTANT SILAGES AND EXUDATES

(Expressed as percentages of dry matter, except dry matter itself, which is percentage of total green weight)

	Stacks.				Pits.			
	1941-42.	1942-43.	1943-44	1944-45.	1941-42.	1942-43	1943-44	1944-45.
Herbage ensiled—								
Dry matter ..	23.51	27.64	20.78	18.50	20.3	27.0	19.2	16.7
Crude protein ..	12.61	12.43	13.27	14.41	13.13	13.76	13.33	15.25
Ether extract ..	2.26	2.26	2.61	2.63	2.73	2.71	3.62	2.44
Crude fibre ..	27.93	28.97	28.31	29.43	25.30	26.23	29.24	42.52
N-free extractives ..	48.83	47.87	46.96	44.46	50.03	48.43	45.05	42.52
Organic matter ..	91.63	91.53	91.15	90.93	91.19	91.13	91.24	90.34
Ash ..	8.37	8.47	8.85	9.07	8.81	8.87	8.76	9.66
Edible silage—								
Dry matter ..	23.50	30.10	22.10	18.80	19.0	23.0	18.2	17.6
Crude protein ..	11.68	12.59	12.10	11.36	12.14	13.44	11.75	11.08
Ether extract ..	2.69	2.82	4.11	4.33	5.01	4.47	6.04	5.72
Crude fibre ..	34.22	31.32	35.91	38.42	33.81	31.07	36.69	38.45
N-free extractives ..	41.95	44.18	39.20	35.54	38.81	40.60	35.39	34.15
Organic matter ..	90.54	90.91	91.32	89.65	89.77	89.58	89.87	89.40
Ash ..	9.46	9.09	8.68	10.35	10.23	10.42	10.13	10.60
pH ..	..	..	..	..	4.68	..	..	..
Inedible silage—								
Dry matter ..	22.0	36.60	32.60	25.00	35.3	24.5	17.0	16.7
Crude protein ..	23.39	20.51	23.14	21.03	*	18.82	18.36	15.05
Ether extract ..	0.88	1.35	0.95	1.27	*	1.70	1.80	2.99
Crude fibre ..	14.98	25.39	17.06	21.84	*	23.89	29.28	31.67
N-free extractives ..	32.50	32.20	30.23	31.34	*	37.53	34.13	32.51
Organic matter ..	71.75	79.45	71.38	75.48	*	81.94	83.57	82.22
Ash ..	28.25	20.55	28.62	24.52	*	18.06	16.43	17.78
Exudate—								
Dry matter ..	..	3.66	5.00	3.49	*	3.56	4.99	3.50
Crude protein ..	..	0.69	*	*	*	2.14	*	*
Ether extract ..	*	..	*	*	*	..	*	*
Crude fibre ..	*	..	*	*	*	..	*	*
N-free extractives ..	*	96.99	*	*	*	96.14	*	*
Organic matter ..	*	97.68	*	*	*	..	*	*
Ash ..	*	2.82	*	*	*	1.72	*	*

\* Not determined.

TABLE V.—LOSSES OF CHEMICAL CONSTITUENTS FROM ENSILAGE IN STACKS AND PITS  
(The differences between the material ensiled and the edible silage produced expressed as percentages of the former)

Constituent.	Stacks.				Pits.			
	1941-42.	1942-43.	1943-44.	1944-45.	1941-42.	1942-43.	1943-44.	1944-45.
Dry matter .. ..	41.5	34.4	41.0	48.7	33.4	25.3	40.4	38.5
Crude protein .. ..	45.8	33.5	46.2	59.6	38.5	27.1	47.5	55.3
Ether extract .. ..	30.4	18.1	7.1	55.5	+22.1	+23.2	0.6	+44.2
Crude fibre .. ..	29.9	29.1	25.1	33.1	11.1	11.5	25.3	21.5
N-free extractives ..	49.8	39.4	50.7	59.0	48.4	37.4	53.2	50.6
Organic matter .. ..	42.2	34.8	41.4	49.4	34.5	26.6	41.3	39.1
Ash .. ..	33.9	29.6	42.1	41.5	22.7	12.3	31.1	32.5

TABLE VI.—APPARENT DIGESTIBILITY COEFFICIENTS OF ENSILED MATERIALS BEFORE AND AFTER ENSILAGE IN STACKS AND PITS

	Stacks.			Pits.		
	1941-42.	1942-43.	1944-45.	1941-42.	1942-43.	1944-45.
Herbage ensiled—						
Dry matter .. ..	74.9	75.4	72.6	74.9	75.4	72.6
Crude protein .. ..	69.9	75.0	75.7	69.9	75.0	75.7
Ether extract .. ..	63.5	59.5	65.5	63.5	59.5	65.5
Crude fibre .. ..	77.7	77.5	73.4	77.7	77.5	73.4
N-free extractives ..	79.9	79.3	74.6	79.7	79.3	74.6
Organic matter .. ..	76.9	75.4	74.2	76.9	75.4	74.2
Ash .. ..	56.3	58.7	57.5	..	..	..
Edible silage—						
Dry matter .. ..	64.2	62.3	60.9	69.9	58.8	64.8
Crude protein .. ..	42.0	33.6	31.2	59.5	30.0	58.4
Ether extract .. ..	58.2	66.7	74.8	69.0	64.8	73.8
Crude fibre .. ..	76.2	69.9	76.8	81.6	66.5	70.9
N-free extractives ..	67.2	65.9	54.6	70.8	57.6	65.8
Organic matter .. ..	67.1	63.1	62.2	73.2	57.2	68.0
Ash .. ..	37.1	48.9	49.4	31.5	45.5	40.4
Average daily ingestion per						
sheep—						
g.						
Herbage .. ..	1,577	1,492	1,371	1,577	1,294	1,371
Silage .. ..	822	701	709	1,213	598	682

TABLE VII.—LOSSES OF DIGESTIBLE NUTRIENTS FROM ENSILAGE IN STACKS AND PITS  
(Differences between the digestible nutrients ensiled and digestible nutrients in the resultant edible silages expressed as percentages of the former)

Digestible Nutrient.	Stacks.			Pits.		
	1941-42.	1942-43.	1944-45.	1941-42.	1942-43	1944-45.
Dry matter ..	49.9	45.8	57.0	38.8	41.8	45.1
Crude protein ..	67.5	70.2	80.8	47.7	70.8	65.5
Ether extract ..	36.1	8.1	3.8	32.2	35.6	61.9
Crude fibre ..	34.6	36.0	30.0	6.6	24.1	24.2
N-free extractives ..	56.8	49.7	70.0	54.1	54.5	56.4
Total organic matter ..	49.7	45.5	57.6	51.9	44.3	44.2
Ash ..	56.5	41.4	49.7	56.7	32.0	52.5

TABLE VIII.—AVERAGES OF PERCENTAGE LOSSES INCURRED IN FOUR YEARS' ENSILAGE IN STACKS AND PITS

	Chemical Constituents.		Digestible Nutrients	
	Stacks	Pits	Stacks	Pits
Dry matter ..	41.4	34.4	50.9	41.9
Crude protein ..	46.3	42.1	72.8	61.3
Ether extract ..	27.8	22.2	16.0	43.2
Crude fibre ..	29.3	17.3	33.5	18.3
N-free extractives ..	49.7	47.4	58.8	55.0
Organic matter ..	41.9	35.4	51.1	46.8
Ash ..	36.8	24.6	49.2	47.1

TABLE IX.—AVERAGE APPARENT DIGESTIBILITIES OF MATERIALS ENSILED AND RESULTANT EDIBLE SILAGES

(Averages of three years' measurements on stack and pit silages)

	Ensiled.	Fifty-ton Stacks	Twenty-five-ton Pits
Dry matter ..	74.3	62.4	64.2
Crude protein ..	73.5	35.6	49.3
Ether extract ..	62.8	66.6	69.2
Crude fibre ..	76.2	74.3	73.0
N-free extractives ..	77.8	62.6	64.7
Organic matter ..	75.5	64.1	66.1
Ash ..	57.5	45.1	39.1

TABLE X.—SUMMARY OF DRY-MATTER LOSSES INCURRED IN SILOS COVERED AGAINST THE WEATHER.  
(All weights given in pounds)

	1941-42.				1942-43.					
	Dry Matter ensiled.	Dry Matter recovered.			Percentage Loss of Edible Dry Matter.	Dry Matter ensiled.	Dry Matter recovered.		Total Loss of Edible Dry Matter.	Percentage Loss of Edible Dry Matter.
		Edible Silage.	Inedible Silage.	Exudate.			Edible Silage.	Inedible Silage.		
Normal stack weighted on top, but no roof weather	23 786	13,908	1,994	*	9,878	18,157	3,697	190	9,516	34.4
Normal stack weighted and roofed against weather	23 404	12,309	1,990	*	11,095	19,213	3,591	28	11,825	38.1
Normal pit weighted, no roof	10 010	6,662	43	*	3 358	13,296	499	287	4,510	25.3
Normal pit weighted and roofed	9,980	7,277	58	*	2,703	12,310	323	316	4,618	27.3

\* Not measured.

TABLE XI.—SUMMARY OF DRY-MATTER LOSSES INCURRED IN SILAGE STACKS WITH AND WITHOUT EARTH CONSOLIDATION.  
(All weights given in pounds)

	1943-44.					1944-45.						
	Dry Matter ensiled.	Dry Matter recovered.			Total Loss of Edible Dry Matter.	Dry Matter ensiled.	Dry Matter recovered.			Total Loss of Edible Dry Matter.	Percentage Loss of Edible Dry Matter.	
		Edible Silage.	Inedible Silage.	Exudate.			Edible Silage.	Inedible Silage.	Exudate.			
Normal stack weighted on top with earth capping	20,787	12,237	2,143	700	8,500	41.0	18,492	9,484	1,608	1,027	9,008	48.7
Stack covered on top and not weighted	22,939	11,699	2,268	569	11,240	49.0	20,622	9,190	1,381	1,168	11,432	55.4

TABLE XII.—SUMMARY OF LOSSES INCURRED IN SILOS WITH AND WITHOUT THE ADDITION OF SULPHURIC-ACID SOLUTIONS  
(All weights given in pounds)

	1943-44.					1944-45.					
	Dry Matter ensiled.	Dry Matter recovered.			Percentage Loss of Edible Dry Matter.	Dry Matter ensiled.	Dry Matter recovered.			Total Loss of Edible Dry Matter.	Percentage Loss of Edible Dry Matter.
		Edible Silage.	Inedible Silage.	Exudate.			Edible Silage.	Inedible Silage.	Exudate.		
Normal pit weighted; no acid	13,430	8,000	421	808	5,430	40-4	11,799	7,258	490	1,272	28-5
Normal pit weighted; 8-3 per cent. H <sup>2</sup> SO <sub>4</sub> solution 1 gallon per 100 lb. green weight	12,608	10,078	487	691	2,530	20-1	12,371	9,002	401	880	27-3
Small experimental pit; no acid	1,236	547	32	59	689	55-8	..	..	..	..	..
Small experimental pit plus acid solution 8-3 per cent.	1,429	1,231	98	35	188	13-9	..	..	..	..	..

## LAND-CRESS TAINT IN CREAM AND BUTTER

### PARTS I AND II

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#### Summary

I. Land-cress (*Coronopus didymus*) is an annual weed which appears in young pastures and in bare patches in established pastures. When consumed by milking-cows it causes the milk and cream to be tainted. The taint is often not noticeable in the cold milk or cream, but appears in the heated product as a "burnt" or "scorched" flavour, which persists in the product after it is cooled. The taint normally is not removable by vacreator treatment. Methods of heating the cream for detection of the taint are described.

II. Land-cress, like garden-cress, contains a benzyl mustard-oil glucoside. Direct steam distillation of the uncrushed plant gave 0.022 per cent. of benzyl cyanide. Steam distillation of the plant after comminution and standing for five hours gave 0.037 per cent. of benzyl cyanide and little evidence of the isothiocyanate. Alcoholic extraction of the glucoside, followed by its separation as insoluble silver salt, and decomposition of this with sodium thiosulphate, gave 0.029 per cent. of oil which was almost pure benzyl isothiocyanate. The latter method was developed to give a quantitative estimation of the isothiocyanate present.

There was little difference in the isothiocyanate content of plants collected at different seasons of the year.

Ether extraction of the minced plant gave 0.014 per cent. of oil which was mainly benzyl cyanide.

Ripened land-cress seeds gave 0.043 per cent. benzyl cyanide by direct steam distillation and 0.065 per cent. of benzyl cyanide by direct steam distillation after grinding and maceration with water. By the silver-salt method the seeds yielded 0.064 per cent. of oil which was mainly benzyl isothiocyanate.

Garden-cress (*Lepidium sativum*) gave 0.022 per cent. of benzyl cyanide by direct steam distillation and 0.03 per cent. of benzyl isothiocyanate by the silver-salt method.

### I. INTRODUCTION

*Coronopus didymus* is a weed known in New Zealand by the popular names "hog-cress," "twin-cress," or, more commonly, "land-cress." (The common name recommended for acceptance in Australia is "lesser swine-cress" (1).) It belongs to the family of Cruciferae and is indigenous to South America. It was first recorded in New Zealand by Raoul in 1846. It occurs widely in Australia, where it has been referred to also as *Senebiera didyma* (2). Davis (3) records it as affecting milk flavour in England.

Land-cress is an annual plant with trailing stems, very finely divided leaves, and small clustered white flowers that are often scarcely visible (Figs. 1 and 2). It flowers over a long period and is constantly producing seeds, which drop off as they ripen. The seeds are carried in a kidney-shaped pod divided into two halves, each of which contains a seed—hence the name "twin-cress." The seeds are scattered abroad by birds. Land-cress plants appear in the early spring in cultivated areas, in open patches in grassland around water-troughs, haystacks, or cowyards where the main pasture



grasses have been trodden out, in paddocks where heavy stocking has resulted in over-grazing and pugging of a pasture leading to a thinning of the sward, in areas thinned out by grass-grub attack, and in patches where the pasture has been weakened during the summer by the growth of large thistle plants. The occurrence of land-cress most troublesome to the dairy-farmer is in areas of newly sown pasture. Young pastures are excellent sources of early spring feed for milking-cows, but their use for this purpose is often



FIG. 1.—Land-cress (natural size).



[Photos from "N.Z. Journal of Agriculture."]

FIG. 2.—Showing spreading habit of growth of land-cress.

prevented by a heavy infestation of land-cress, the seed of which readily germinates in the patches of bare ground in the open young pasture. Spring-sown pastures, especially following a crop of maize, which provides conditions for the prolific seeding of cress plants, suffer the heaviest infestations, but the cress plants may appear in abundance in autumn-sown young pastures. Ailo(4) has observed the presence of 502 cress plants in one square yard of a spring-sown pasture, the average of twenty-four counts being 106 plants per square yard.

The land-cress plant when crushed between the fingers has a strong odour, and in the mouth a strong sharp burning taste somewhat resembling, but less clean and appetizing than, that of garden-cress. The odour is noticeable on a warm day in the atmosphere above a heavily infested area. When the plants are consumed by milking-cows a strong flavour may be imparted to the milk and to the dairy product manufactured from it :—

- (a) *Milk or Cream*.—Cress flavour is not readily detected in raw milk or cream, but becomes much more apparent after pasteurization at a high temperature. The reason for this has not been clearly established. The flavour is readily apparent in the cooled pasteurized cream.
- (b) *Cheese*.—Riddet and Valentine(5) have reported on the effect of land-cress on cheese flavour. Cress flavour was observed in the milk on six out of eight occasions when it was subsequently observed in the cheese. It was noticeable during, and still persisted after, flash-pasteurization of the cheese milk. The flavour in the immature cheese was described as “pungent” and “biting,” and was more noticeable in cheese of high acidity than in cheese of normal acidity. The flavour in the mature cheese was described by the Department of Agriculture Graders in London as “unclean,” “scorched,” or “herby.”
- (c) *Butter*.—The land-cress flavour in butter was formerly described as “burnt” or “scorched,” and was attributed to overheating during pasteurization. Now that the source of the taint has been recognized, the flavour in the butter is usually described simply as “cress.” Land-cress taint causes more trouble in the butter-factory than in the cheese-factory, because the flavour is more easily apparent in butter than in the naturally more strongly flavoured cheese, and also because of the higher cream-pasteurization temperatures which accentuate the flavour. The occurrence of strong cress taint in butter has a marked effect on the grade score, resulting in considerable loss to the butter-factory. The tainting effect is so strong that 2 gallons or 3 gallons of tainted cream can affect the whole of the butter from a 3,000-gallon vat of cream.

#### DETECTION OF LAND-CRESS TAINT

Land-cress taint, unless very strong, is difficult to detect in the grading of cold cream, and frequently escapes the notice of the cream-grader. A small quantity of cress-tainted cream can taint a very large volume of good cream, and the first evidence of the presence of land-cress taint in the cream may be in the hot vapours rising from the steaming of cream from the can or from the top of the open-surface water cream-cooler. It is then too late to segregate the cream-supply carrying the taint. In factories where the cream-cooler used is of the closed type, as, for example, a plate cooler, or where the cream is drawn from the cooler unit of the vacreator at a low temperature, the presence of cress taint in the pasteurized cream may pass unnoticed, and the first intimation of trouble with cress taint may be the Grader's report on the butter. Cress taint may, however, be noticeable in the warm water issuing from the first condenser-ejector of the vacreator. When cress taint is noticed in the pasteurized cream or in the butter, the usual practice in New Zealand factories is to heat a sample of each supply

of cream before the cream is graded and "tipped," so that the cream carrying the taint can be segregated. Several systems of heating rapidly before grading have been in use:—

- (a) Heating of a sample in a butter-moisture basin on a steam plate located near the cream-grader. In factories receiving cream at full speed, this method is too time-consuming.
- (b) A small vessel with running hot-water supply attached is kept beside the grader. A sample of cream is thrown on to the surface of the hot water. The full cress taint is immediately developed by the heat and is readily detectable.

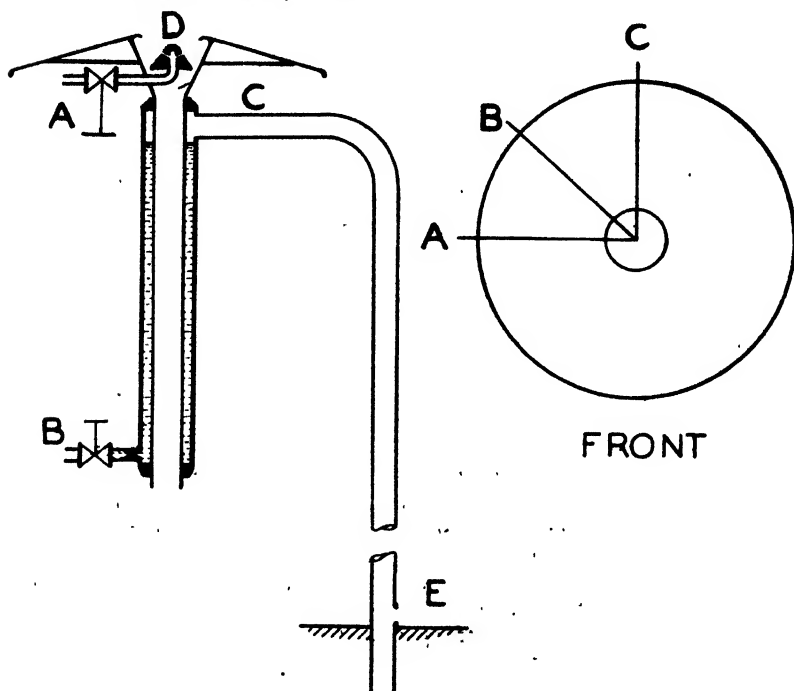


FIG. 3.

Diagram of Murray hot tester for use in the grading of cream. Tester consists of a 12 in. length of 1 in. diameter tinned copper tube fixed by means of soldered brass rings inside a circular length of 2 in. plated brass tubing. The outside tube has a steam inlet connection and valve at B, and a 1 in. outlet C connected to  $\frac{1}{2}$  in. galvanized pipe which serves as a stand to hold the tester at a convenient level from the ground. The top of the copper pipe is connected to a copper funnel which terminates in an incurved top as shown, and is surrounded by a wide spreading cover. D is a small conical spreader, supported in the centre of the funnel by a  $\frac{1}{2}$  in. copper tube connected to a water-tap. The spreader is a tinned brass cone pierced by four  $\frac{1}{4}$  in. holes bored so that the stream of water issuing from them will strike just under the curved part of the top cover. The copper tube just below the spreader also has four holes staggered in position in relation to the holes through the spreader, and bored so that the stream of water from them strikes the under-surface of the spreader and is diverted downwards.

The jacket is filled with water at about 180–190° F. A spoonful of the cream to be graded is thrown on to the spreader, which distributes it over the lower portion of the funnel when it runs down over the hot tubes. The natural up-draught of air carries the vapours out of the top of the tube, where the cress can be detected by the odour. The tap A is then turned on for a few seconds to wash the cream to waste.

- (c) A special "hot tester," as developed by Mr. H. Lamont Murray, is installed beside the grader (see Fig. 3). A spoonful of cream is thrown on to the tester, and the vapours rising from the cream are carried upwards by the natural draught created by the heat. The device can be flushed with water after each grading.
- (d) A "hot tester" developed by Mr. Ward, manager of New Zealand Co-operative Dairy Co., Te Awamutu Branch (see Fig. 4).

In the butter-factory the normal process of treatment of cream by steam distillation in the vacreator, which suffices to remove a number of off-flavours from the cream, will not remove land-cress taint. Part II of this series describes attempts to define the chemical nature of the substance or substances causing land-cress taint, and later papers will describe attempts to devise means for removal of the taint from tainted cream. Contemporaneous with this work a study has been made of the effect of controlled grazing of cows on infested pastures on the incidence of cress taint in the milk and cream (Part III of this series). The work has been greatly hampered

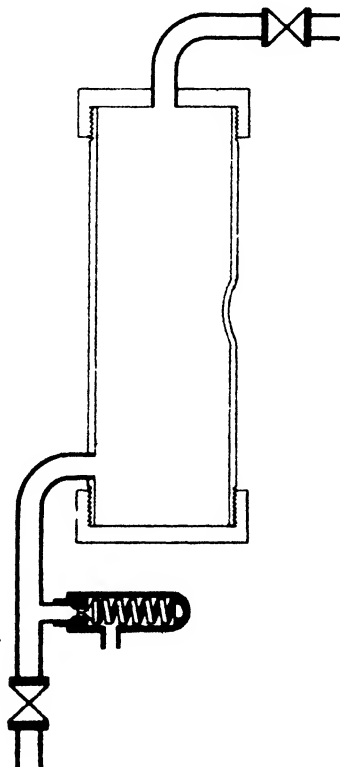


FIG. 4.

Hot tester for detecting land-cress taint in cream, consisting of a small length of 3 in. diameter stainless steel tubing, fitted with a union plug at each end, and with steam connections and safety valve as shown. The 3 in. pipe has a small indentation at the top. The cream to be graded is placed in this indentation and, with steam turned on to the unit, rapidly reaches boiling-point. The device is easily cleaned by wiping with a damp cloth or, if necessary, scrubbing by means of a steel "mit."

by the difficulty of obtaining reliable assessments of the strength of cress taint. The grading of one sample of tainted cream or butter leaves a residual after-effect, which tends to disturb the grading of later samples. Procter(6) found with camomile (mayweed) taint that, after tasting one sample of tainted milk, one cannot rely on one's sense of taste. Without a satisfactory means of obtaining even a reliable rough quantitative comparison it has been difficult to make the desired progress with the work. In this respect elucidation of the chemical nature of the tainting material should be of great assistance.

## II. THE ESSENTIAL OILS OF LAND-CRESS (*CORONOPUS DIDYMUS*) AND OF GARDEN-CRESS (*LEPIDIDIUM SATIVUM*)

Land-cress contains a mustard-oil glucoside and also a myrosinase which can cause fission of the glucoside, setting free the mustard-oil, when the leaves are crushed to give contact between glucoside and enzyme. The chemical nature of the glucoside in land-cress has not previously been studied, but information is available on the composition of the glucosides of a number of other cresses. Garden-cress (*Lepidium sativum*) and Indian cress or ordinary garden nasturtium (*Tropaeolum majus*) contain the benzyl mustard-oil glucoside (Gadamer(7)). Watercress (*Nasturtium officinale*) and wintercress (*Barbarea praecox*) contain the phenyl ethyl mustard-oil glucoside (Gadamer, *loc. cit.*). Scurvy-grass (*Cochlearia officinalis*) contains the sec.-butyl mustard-oil glucoside. Hoffman(8) steam distilled watercress plants and obtained an essential oil consisting mainly of phenyl propionyl nitrile. Gadamer(7) found that when finely comminuted watercress plants were steam distilled, an essential oil was obtained consisting of phenyl ethyl isothiocyanate, whereas steam distillation of the whole—i.e., uncrushed—plant gave the nitrile as found by Hoffman. Similarly, when the seeds of garden-cress were steam distilled after grinding they yielded an essential oil consisting mainly of benzyl isothiocyanate (Gadamer(7)), whereas when they were steam distilled without grinding the essential oil consisted almost entirely of benzyl cyanide. Gadamer attributed the difference to the effect of grinding or mincing in bringing the myrosinase enzyme into contact with the glucoside, so setting free the isothiocyanate, whereas direct steam distillation caused a different fission of the glucoside, resulting in the formation of the nitrile. Ground *Tropaeolum* seeds also yield benzyl mustard-oil on steam distillation(7). Gadamer attempted to isolate the benzyl mustard-oil glucoside from garden-cress and *Tropaeolum* seeds, but obtained only a syrup containing 25 per cent. to 30 per cent. of the glucoside.

### ISOLATION OF THE ESSENTIAL OIL OF LAND-CRESS

#### (1) Direct Steam Distillation of the Uncrushed Plant

The material used consisted of large cress plants, still fresh and green, but with the characteristic long trailer vines and some flowers and seeds. A total quantity of 140 lb. in four lots was distilled with steam from a separate boiler. Distillation was accompanied, in the early stages particularly, by copious evolution of sulphur-containing gases. The distillate was turbid and showed a small amount of free oil. The oil was removed in a separating-funnel and the aqueous portion was extracted twice with petroleum ether (b.p. 40–60° c.). From the distillation of each 35 lb. of cress, 15 l. of distillate were collected. The separated oil was added to the petroleum-ether extract and was dried over sodium sulphate. The petrol ether was removed on the water bath and finally under reduced pressure, leaving 13.9 g. of a brownish-coloured oil,  $n_D^{20}$  1.5237,  $d_{15}^{15}$  1.019. The yield of oil was 0.022 per cent. The crude oil contained both sulphur and nitrogen, and also a small amount of crystalline material which was filtered off. Distillation of the oil under reduced pressure gave three fractions:—

- |                    |                          |
|--------------------|--------------------------|
| 1. -103°/10 mm.    | 3 g., $n_D^{20}$ 1.5215. |
| 2. 103–105°/10 mm. | 6 g., $n_D^{20}$ 1.5230. |
| 3. 105–108°/10 mm. | 2 g., $n_D^{20}$ 1.5232. |

Sulphur was present in fraction 1, but not in fractions 2 and 3. The constants of the oil agreed closely with those for benzyl cyanide (b.p.  $107^{\circ}/12$  mm.  $n_D^{25}$  1.5210,  $d_{15}^{15}$  1.021) (10), and hydrolysis of a portion of fraction (2) with sulphuric acid gave phenylacetic acid m.p.  $73^{\circ}$  c. (correct for phenylacetic acid  $76^{\circ}$  c.). The oil obtained by direct distillation of uncrushed land-cress plants was thus mainly benzyl cyanide. The crystals separated from the oil were found to be phenylacetic acid.

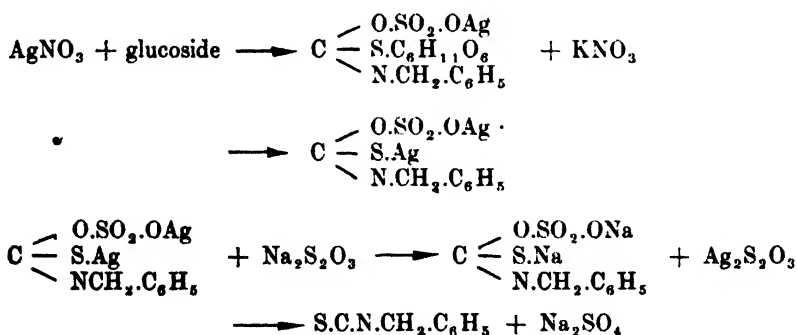
Cress plants from the Bay of Plenty district, at a time when trouble from cress taint in cream was very prevalent, gave a similar yield of oil consisting of benzyl cyanide.

### (2) Direct Steam Distillation of the Commminuted Plant

The plant material (4.3 kg.) was passed through a chopper and then through a mincer, which caused exudation of most of the plant juice. The juice was added to the finely divided plant and was left in contact for five hours. The material was then transferred to a 10 l. flask and distilled in steam for two hours. Three litres of distillate were collected. The oil suspended in the distillate was extracted with petroleum ether as before. Yield of oil, 1.60 g., 0.037 per cent. The oil contained a small amount of crystalline material, which was filtered off. The refractive index was  $n_D^{20}$  1.5300,  $d_{20}^{20}$  1.032. The oil from the finely divided plant material was thus also mainly benzyl cyanide.

### (3) Indirect Isolation of the Isothiocyanate

Since it was not found possible by direct steam distillation to obtain the isothiocyanate from the land-cress plant, an indirect method was employed using the sequence of reactions described by Gadamer(7), who showed that mustard-oil glucosides form insoluble silver salts which are decomposed by sodium thiosulphate to yield the free mustard-oil -



The uncrushed cress plants (20 kg.) were steeped in boiling alcohol to destroy enzymes. They were then sucked dry of alcohol in a Buchner funnel and were passed through a mincer. The minced material was extracted with two further quantities of hot alcohol. The alcohol solution was allowed to stand for some time, and the supernatant clear portion was siphoned off. The lower portion, which consisted of a sludge of plant tissue, was centrifuged and the clear alcoholic layer was combined with the portion separated by siphoning. Silver nitrate (1,500 ml. of 20 per cent. solution) was added

to the combined alcoholic extracts, yielding a copious precipitate of a dark-coloured silver salt which was allowed to settle overnight. The clear supernatant liquid was siphoned off and the silver salt was separated from the remainder in a centrifuge. The cake of silver salt was washed twice by mixing with water in the centrifuge tubes, and was then decomposed by addition of sodium thiosulphate (1,500 ml. of 40 per cent. solution). A strong odour of mustard-oil was immediately apparent. Previous trials had shown that direct steam distillation in presence of the sodium thiosulphate resulted in destruction of the mustard-oil. The mustard-oil was therefore extracted several times with ether. Care in shaking was necessary, to avoid the development of intractable emulsions. Direct fractional distillation of the residue from the ethereal extract had been found to give trouble through undue frothing and some decomposition. The residue was therefore steam distilled. The distillate contained a visible oil which was separated in a separating-funnel. The aqueous portion of the distillate was extracted three times with petroleum ether, and the extract was combined with the separated oil. Removal of the solvent, after drying over sodium sulphate, left 5.8 g. of a clear oil with a sharp radish odour. The yield was 0.029 per cent.,  $d_{20}^{20}$  1.123,  $n_D^{20}$  1.6010, b.p. 121–140°/12 mm. The constants agree closely with those of benzyl isothiocyanate ( $d_4^{15}$  1.125,  $n_D^{20}$  1.6035, b.p. 121–125°/12 mm.), and the main fraction, warmed with alcoholic ammonia, gave a thiourea m.p. 162–163° C. (m.p. of benzyl thiourea, 165° C.).

It is clear that land-cress resembles garden-cress in containing a benzyl mustard-oil glucoside.

#### QUANTITATIVE ESTIMATION OF BENZYL ISOTHIOCYANATE IN THE CRESS PLANT

The effect of ingestion of land-cress taint by the cow on the flavour of cream appears to vary with the season. It therefore became important to know whether there was any seasonal variation in the amount of mustard-oil glucoside present. For this purpose, the method of isolation of the mustard-oil through the silver salt was carried out on a quantitative basis. The method was checked as follows:—

Six 250 g. quantities of uncrushed land-cress were each steeped in 600 ml. of boiling alcohol, and the alcohol was separated as before. The cress-plants were passed through a juice-extractor, which crushed out most of the juice. The solid residue was extracted with a further 300 ml. of hot alcohol. For purposes of checking the accuracy of the method of isolation of the isothiocyanate, the six extracts were combined and divided again into six equal portions. Each of these portions, after being treated separately with 150 ml. of 10 per cent. silver nitrate solution, was centrifuged for separation of the silver salts. The silver salts were decomposed with 200 ml. of 20 per cent. solution of sodium thiosulphate. The isothiocyanate was extracted with 1 l. of ether and was steam distilled, 300 ml. of distillate being collected. The condenser was then washed down with petrol ether, and the distillate extracted twice with 300 ml. of petrol ether. The petrol-ether solution was dried over sodium sulphate. Three methods of estimation of the isothiocyanate were then applied in duplicate to the six quantities of residual oil obtained: (a) *direct weighing of the oil after removal of petrol ether to constant weight under reduced pressure*; (b) *estimation of nitrogen by the Kjeldahl method* (previous trials had shown that allyl thiocyanate could be

accurately estimated by the Kjeldahl method); (c) *quantitative conversion to the benzyl thiourea* by heating with alcoholic ammonia and weighing the thiourea produced. The following results were obtained:

- (a) By direct weighing—  
0.1578 g. oil = 0.063 per cent. } Mean, 0.065 per cent.  
0.1668 g. oil = 0.067 per cent. }  
(b) By Kjeldahl estimation of nitrogen in oil isolated under (a) —  
0.069 per cent. } Mean, 0.064 per cent.  
0.058 per cent. }  
(c) By conversion to thiourea  
0.1948 g. thiourea = 0.070 per cent. } Mean, 0.067 per cent.  
0.1760 g. thiourea = 0.063 per cent. }

The degree of agreement shows that the isolation of isothiocyanate from the plant glucoside solution gave reasonably uniform results over the six extractions and that the three methods of estimation were equally applicable. In subsequent work, estimations were made by direct weighing of the oil, followed by a Kjeldahl estimation of nitrogen as confirmation of the complete removal of petrol ether and of the composition of the oil as mainly the isothiocyanate.

#### ISOTHIOCYANATE CONTENT OF THE LAND-CRESS PLANT AT DIFFERENT SEASONS OF THE YEAR AND AT DIFFERENT STAGES OF GROWTH OF THE PLANT

As will be shown in a later paper of this series, the effect of ingestion of land-cress by the cow on the incidence of land-cress taint in the milk or cream is not uniform. Many instances can be quoted where land-cress has been consumed by cows without the appearance of the taint in the milk or cream. Ingestion of the plant produces the taint more regularly during the spring months than later, and after the end of December in most seasons the plant seems to be fairly innocuous to the quality of the cream and butter (this was not so during the 1945-46 season). In dry seasons the plant reaches maturity more quickly and further germinations of seed do not take place. Hence plants consumed by cows during the late summer and autumn months are likely to be long, stalky, and fibrous, compared with the fresh young leaves of the early spring growth. By use of the rough quantitative method described above, comparisons have been made of the isothiocyanate content of the plants at various stages of growth and at various times during the season.

(a) *Fresh Young Plants compared with Larger Older Plants containing Fibrous Stems, taken at the same Time.* —The results were:—

Description of Plants.	Isothiocyanate Content.		
	By weighing of Oil Isolated (per Cent.).	By Nitrogen Estimation on the Oil (per Cent.).	Average (per Cent.).
Small fresh plants .. ..	0.045	0.043	0.044
Large plants .. ..	0.059	0.053	0.056



There was thus a larger content of glucoside in the older plants. As these latter would probably contain a smaller proportion of water, the difference would scarcely be significant on a dry-matter basis.

(b) *Mature Dry Stalky Plants collected in January.*—The isothiocyanate content was found to be 0.034 per cent.

(c) *Mixture of Fresh and Mature Samples collected in March during a Wet Season (1946).*—The isothiocyanate content was found to be 0.045 per cent.

There was no significant difference in the isothiocyanate content of the plants at the different stages of growth.

#### ETHER EXTRACTION OF THE MINCED PLANT

Four kilogrammes of fresh plant collected in March, 1946, were minced and then macerated with water for eight hours. The aqueous portion was separated from the plant material by filtration, and was extracted several times with ether. The ether was removed, and the residue, still containing chlorophyll, was steam distilled. Petrol-ether extraction of the distillate gave 0.014 per cent. of oily residue,  $n_D^{20}$  1.5159. The extract was thus mainly benzyl cyanide. A second trial gave 0.014 per cent. of oil,  $n_D^{20}$  1.5401, with an odour of both benzyl cyanide and benzyl isothiocyanate, in keeping with the indication of the refractive index.

#### RIPENED LAND-CRESS SEEDS

##### (a) *Alcoholic Extraction*

Ripened land-cress seed was ground in a Wiley mill to give a coarse powder in which all the seeds were broken. The powder (5 kg.) was extracted twice, over one and one-half hours, with 3 l. of hot alcohol, the alcohol was removed each time through a Buchner funnel under reduced pressure, and the residue was washed with hot alcohol. The extract (8 l.) was clarified, first by decantation after two days' standing, and finally by centrifuging the sludge. The clear extract was used for estimation of isothiocyanate as detailed above. In this way 3.2 g. of golden-yellow oil were obtained,  $n_D^{20}$  1.5980,  $d_4^{20}$  1.126. The oil was thus mainly benzyl isothiocyanate, the yield being 0.064 per cent.

##### (b) *Direct Steam Distillation*

Two and a half kilogrammes of seeds were steam distilled directly without grinding. Estimation of the distillate with petrol ether as before gave 1.08 g. (0.043 per cent.) of oil,  $n_D^{20}$  1.5204. The oil thus was mainly benzyl cyanide.

##### (c) *Direct Steam Distillation of the Ground Seeds after Maceration in Water*

Two and a half kilogrammes of seeds were ground in a Wiley mill. Water was added to cover the seed, and the mixture was allowed to stand for six hours. Extraction of the distillate gave 1.69 g. (0.068 per cent.) of oil,  $n_D^{20}$  1.5215. The oil thus was almost wholly benzyl cyanide.

#### ESSENTIAL OIL OF GARDEN-CRESS

During the course of the work on land-cress taint in cream in relation to butter quality it became necessary to compare the effects of ingestion of land-cress and of garden-cress by the cow on the flavour of cream and butter.

Simultaneously with these feeding trials, the nature and content of the essential oil in the garden-cress material used was estimated by methods similar to those described above for land-cress:—

- (1) *Steam Distillation of the Uncrushed Plant.*—Four and a half kilogrammes in a 10 l. flask gave 0.99 g. oil, yield 0.022 per cent.,  $n_D^{20}$  1.5219,  $d_{20}^{20}$  1.021. The oil was therefore benzyl cyanide in agreement with the findings of Hoffman(4) for steam distillation of garden-cress.
- (2) *Steam Distillation of Comminuted Garden-cress.*—(Garden-cress (4.5 kg.) was comminuted in a mincer and allowed to stand for eight hours. Steam distillation gave a turbid distillate, but with no visible oil droplets. Extraction of the distillate gave 0.16 g. oil, yield 0.003 per cent. The oil gradually solidified, and was obviously not benzyl isothiocyanate. The finding of Gadamer that steam distillation of finely divided garden-cress seeds gave benzyl isothiocyanate was thus not confirmed for the fresh plant material.
- (3) Extraction of 9 kg. of garden-cress plant with hot alcohol, and treatment with silver nitrate and sodium thiosulphate as described above, gave 2.67 g. of a clear yellow oil, yield 0.030 per cent.,  $n_D^{20}$  1.6003,  $d_{20}^{20}$  1.124. The oil had the odour and the physical constants of benzyl isothiocyanate and gave benzyl thiourea with alcoholic ammonia.

It is apparent, therefore, that land-cress and garden-cress yield approximately the same proportions of benzyl cyanide on direct steam distillation, and of benzyl isothiocyanate by the silver-salt method. The only difference noted was the peculiar "burnt" odour of the land-cress oil obtained by direct distillation. This odour was not present in the oil obtained by the same method from garden-cress.

#### ACKNOWLEDGMENT

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## THE THIAMIN, RIBOFLAVIN, AND NIACIN CONTENT OF SOME NEW ZEALAND MILKS

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### Summary

Thiamin was estimated by the fluorimetric method, and riboflavin and niacin were estimated microbiologically. Thiamin and riboflavin contents of Jersey milks were regularly higher than those of Friesian milks, but niacin content was independent of the breed of cow.

Feeding of meals to cows on pasture appeared to cause a rise in thiamin content of the milk from Jersey cows, but not from Friesian cows. The riboflavin and niacin contents of the milks were not affected.

Wintering of cows under poor conditions did not affect the thiamin, riboflavin, and niacin contents of the milks as compared with wintering under good conditions, when the two groups were kept under the same feeding conditions during lactation.

The samples analysed represent composites from groups of four to twelve cows.

The thiamin content of Jersey milk varied from 0.032 mg. to 0.088 mg. per 100 ml., average over the six groups, 0.0481 mg. per 100 ml.; and of Friesian milk, from 0.029 mg. to 0.056 mg. per 100 ml., average over the four groups, 0.0414 mg. per 100 ml.

The riboflavin content of Jersey milk varied from 0.19 mg. to 0.33 mg. per 100 ml., average over the six groups, 0.255 mg. per 100 ml.; and of Friesian milk, from 0.14 mg. to 0.28 mg. per 100 ml., average over the four groups, 0.194 mg. per 100 ml.

The niacin content of the milks varied from 0.08 mg. to 0.15 mg. per 100 ml., average over all the groups, 0.10 mg. per 100 ml.

THE content of water-soluble vitamins in cow's milk has already been studied in other countries, but results are not available for freshly produced New Zealand milks. The present paper gives the results for thiamin, riboflavin, and niacin estimations in milks from several separate sections of the herds at Massey Agricultural College and the Dairy Research Institute (N.Z.) during the years 1944 to 1946. The groups were selected for various trials in general dairy husbandry practice, which will be reported on elsewhere. Since these trials called for the regular weighing, sampling, and analysis of the milks from each cow in each group, the opportunity was taken to collect group samples for vitamin estimation.

### SAMPLING AND ANALYSIS

The milk from each group of cows was sampled at the morning milking. Houston *et al*(1) found that the total thiamin content of milk did not vary greatly with variation of the period between milkings, and Theophilus and Stamberg(2) have shown that the riboflavin content of morning, noon, and night milks taken on the same day was constant within the limits of experimental error. A small amount of the production of each cow was

collected in an amber-coloured sample bottle to give a proportionate sample for the group. Samples were taken once fortnightly throughout the lactation period and were analysed immediately by one of us (N. O. B.) at the Plant Chemistry Laboratory.

Yields of milk and fat contents of the milk were measured twice weekly. From these figures the yearly average production of milk and butterfat, and so the yearly average fat test of the milk, were calculated. Similarly, values for the yearly average vitamin content of the milks of each group have been calculated from the total milk production for each fortnight (one week on each side of the sampling date) and the vitamin content of the milk.

#### DESCRIPTION OF THE SEPARATE GROUPS OF COWS

*Series I.*—Two paired groups (A and B), each of twelve Jersey cows calving in the spring months, and two similar groups (E and F), each of four Friesian cows, were fed : -

Group A : On normal pasture, with the usual hay, silage, green feed, or root supplement as available and as required.

Group B : On the same feeds as for Group A, with, in addition, some meals at each milking.

Group E : As for Group A.

Group F : As for Group B.

It was assumed, as an average working basis, that the feeds supplied from normal sources were sufficient for body maintenance and for production of 2 gallons of Jersey and of 3 gallons of Friesian milk. For production beyond this level, meals were fed to Groups B and F at a rate of 4.5 lb. per gallon of Jersey milk and 3.8 lb. per gallon of Friesian milk, with a minimum amount of 4 lb. per cow per day.

The meal used had the following composition (figures in percentages) : barley-meal, 20 ; wheat-meal, 20 ; bran, 20 ; oats, 10 ; pea-meal, 5 ; linseed-cake, 25. The calculated starch equivalent of the meal was 66.

*Series II.*—The trial was similar in scope to the Series I trial, but the cows were autumn calvers and they were fed meals only during the period of winter scarcity of feed—viz., from May until 19th October. Each group consisted of four cows, as follows :

Group C : Four Friesian cows on ordinary diet.

Group D : Four Friesian cows on ordinary diet plus meals.

Group E : Four Jersey cows on ordinary diet.

Group F : Four Jersey cows on ordinary diet plus meals.

*Series III.*—Two paired groups, each of eight Jersey cows, were used. One group (HP) was fed on a high plane of nutrition during the winter dry period and came into profit in good condition. The second group (LP) was fed on a low plane of nutrition during the dry period until calving-time and came into profit in relatively poor condition. Thereafter throughout the lactation period the two groups were kept under exactly similar feeding conditions.

## METHODS OF ESTIMATION OF VITAMIN CONTENT

**Thiamin.**—Ten millilitres milk and 20 ml. 0.25N-HCl were autoclaved for fifteen minutes at 15 lb. pressure. After cooling, the extract was brought to pH 4.5, 0.25 g. clarase was added, and the mixture was incubated at 37° C. overnight. The contents of the flask were then made to 50 ml. and centrifuged, and the clear aqueous portion was used for the analysis. The fluorimetric method of Hennessy and Cerecedo(3) was used, the absorption step being omitted. The fluorescence was measured visually by the method of Harris and Wang(4).

**Riboflavin.**—Five millilitres milk were autoclaved with 50 ml. 0.25N-HCl in an amber glass flask for fifteen minutes at 15 lb. pressure. After cooling, the extract was brought to pH 6.8, made up to 100 ml., and centrifuged. The clear aqueous portion was taken for the assay, for which the microbiological method of Snell and Strong(5) was used. The extracts were at all times protected from excessive light.

**Niacin.**—The extracts were prepared exactly as for riboflavin, except that protection from light was unnecessary. The method of assay (microbiological) was that of Snell and Wright(6).

## RESULTS

The yearly average thiamin contents of the milks, calculated from the fortnightly milk-production and thiamin-content figures, are shown for the three groups in Table I, and the fortnightly results are plotted for the three groups in Fig. 1.

TABLE I.—YEARLY AVERAGE, MINIMUM AND MAXIMUM THIAMIN CONTENTS OF MILKS FROM EACH GROUP OF COWS IN THE THREE SERIES

Year.	Series.	Group.	Breed of Cow.	Number of Cows.	Number of Analyses.	Yearly Average Fat Test	Thiamin Content (Milligrams per 100 ml.)		
							Yearly Average.	Minimum	Maximum
1944-45	I (spring calvers)	A	Jersey	12	20	5.17	0.041	0.032	0.051
		B	Jersey	12	20	4.99	0.046	0.032	0.056
		E	Friesian	4	20	3.44	0.039	0.032	0.051
		F	Friesian	4	20	3.51	0.038	0.029	0.048
1945-46	II (autumn calvers)	C	Friesian	4	20	3.71	0.044	0.040	0.048
		D	Friesian	4	20	3.71	0.044	0.038	0.056
		E	Jersey	4	20	5.45	0.055	0.045	0.075
		F	Jersey	4	20	5.53	0.059	0.048	0.088
1945-46	III	HP	Jersey	8	17	5.28	0.049	0.042	0.058
		LP	Jersey	8	17	5.40	0.051	0.043	0.064

**Average Values.**—The average values for thiamin content are of the same order as those quoted for milks in other countries. Sherman, for example(7), quotes the range of values within which the average for milk will probably be found as 0.040 mg. to 0.065 mg. per 100 g., and the Bureau of Human Nutrition and Home Economics of the United States Department of Agriculture, in co-operation with the United States National Research Council(8), quote the average value for whole milk as 0.04 mg. per 100 g. Ritter(9) quotes 0.048 mg. per 100 g. for Swiss whole milk. Holmes *et al*(10) found the average value for late summer milk from a mixed herd of cows to be 0.044 mg. per 100 ml., Kendall(11) found values for raw milk ranging from 0.041 mg. to 0.048 mg. per 100 ml., and Pearson *et al*(25) reported an average value of 0.38 mg. per 100 ml.

## EFFECT OF FEEDING CONDITIONS ON THIAMIN CONTENT

The supplementary feeding of meals to Friesian cows had no apparent effect on the thiamin content of the milk, either during summer or winter

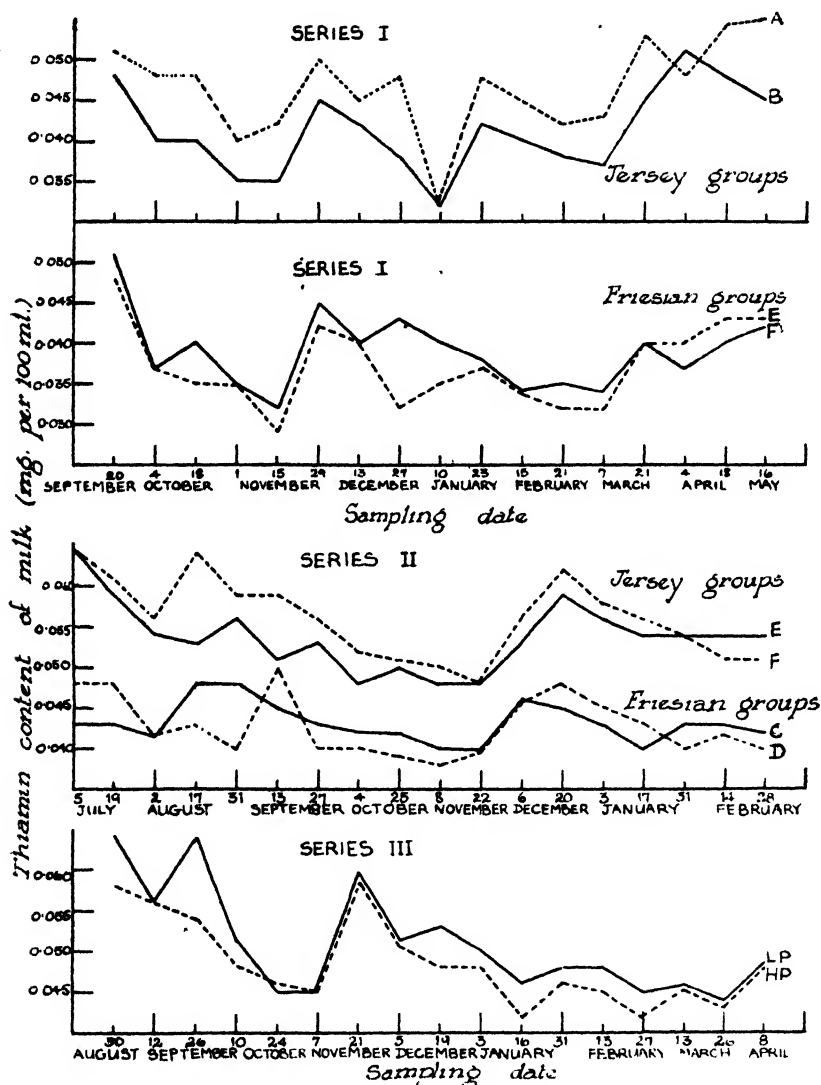


FIG. 1.—Thiamin content of mixed milks from each group of cows in the three series of trials.

Series I and II .. ——— Cows fed on pasture.  
 .. - - - Cows fed on pasture plus meals.  
 Series III .. ——— Cows wintered on low plane of nutrition.  
 .. - - - Cows wintered on high plane of nutrition.

feeding conditions (Series I, E and F; Series II, C and D; Table 1; and Fig. 1). The milks from both groups of Jersey cows given a supplement of meals, on the other hand (Series I, B, and Series II, F), had higher thiamin

content than the milks from the corresponding groups of Jersey cows on ordinary diet. The difference in yearly average thiamin content of the milk was 0.005 mg. and 0.004 mg. per 100 ml. respectively. The difference in the yearly average thiamin content for Series I is a reflection of differences persisting throughout the lactation period. The difference for the Series II results was greater during the early portion of the lactation—i.e., at the time when the supplementary feed was given—than in the later portion of the lactation following the cessation of supplementary feeding on 20th October. It does appear, therefore, that the feeding of meals to Jersey cows on pasture caused a rise in thiamin content of the milk. The reason for the absence of effect of the supplementary feeding on the thiamin content of Friesian milk is not apparent from the trials. Kon(12), reviewing the literature on the subject, states that the thiamin content of cow's milk is remarkably independent of the feed, and that thiamin in all probability is formed in the rumen by bacterial synthesis. Holmes *et al*(13) found no difference following transference of cows from dry feeds to rapidly growing young pasture, but Houston *et al*(1) found higher values for a group of Short-horn cows fed indoors on dry feed than for a similar group at pasture.

In the Series III trials there was only a slight difference (0.002 mg. per 100 ml.) in the yearly average thiamin content of the milks from the two groups of cows, but this difference persisted throughout the greater portion of the lactation period. The higher values were obtained for the milks from the cows wintered under poor feeding conditions. The milks from the "low plane" groups had the higher yearly average percentage fat content, and the difference in thiamin content may be related to this factor, or may actually be an effect of the difference in feeding conditions, or may be due to random individual cow differences in so small a group. Further work would be necessary before any firm conclusions are possible as to the regular existence of a difference in thiamin content due to the difference in conditions of wintering the animals during the dry period.

#### EFFECT OF STAGE OF LACTATION ON THIAMIN CONTENT

The graphs in Fig. 1 show that in Series I trials there was a general fall in thiamin content of the milk in the first month of lactation and a general rise during the last two months, but no general trend during the intervening months. In Series II trials the fall in the early stages of lactation occurred only in the milk from the Jersey groups, and there was no rise at the end of lactation. The Series III results show the fall at the beginning of lactation, but no rise at the end of lactation. Houston *et al*(1) found a sharp fall in thiamin content of milk during the first thirty to sixty days of lactation, and a slight rise towards the end of lactation (figures milligrams per 100 ml.), as follows: colostrum (excluding the first colostrum), 0.06–0.10; early-lactation milk, about 0.06; mid-lactation milk, 0.030–0.035; late-lactation milk, 0.030–0.040. Pearson *et al*(25) found that the high thiamin content of the milk after parturition persisted for more than ten days.

#### EFFECT OF BREED OF COW ON THIAMIN CONTENT

Comparisons of the thiamin content of milks from Jersey and Friesian cows kept under similar feeding conditions are available from the results in both Series I and Series II trials. The yearly average thiamin contents of the Jersey and Friesian milks were: Series I, 0.0435 and 0.0385,

difference 0.005; and Series II, 0.057 and 0.044, difference 0.013. The Jersey milk thus had a higher yearly average thiamin content than the Friesian milk. This difference was persistent throughout the lactation period, as is evident from the graphs in Fig. 1. The reason for the much higher yearly average values for both the Jersey and the Friesian milks in Series II trials as compared with the Series I trials is not established. The yearly average fat tests of the Series II milks were higher, but the differences are not so great as the proportional differences in thiamin content.

#### SUMMARY OF DISCUSSION ON THIAMIN CONTENT OF MILK

The thiamin content of the New Zealand milks examined was within the range of values quoted for milks by overseas workers. Supplementary feeding of meals to cows at pasture increased the thiamin content of Jersey milk, but not of Friesian milk. Jersey milk had a higher content of thiamin than Friesian milk.

#### RIBOFLAVIN

The yearly average riboflavin content of the milks, calculated from the fortnightly milk-production and riboflavin-content figures, are shown for the three groups in Table II. The average values for the different herds are of

TABLE II.—YEARLY AVERAGE, MINIMUM AND MAXIMUM RIBOFLAVIN CONTENTS OF MILKS FOR EACH GROUP OF COWS IN THE THREE SERIES

Year.	Series.	Group.	Breed of Cow.	Number of Cows.	Number of Analyses.	Riboflavin Content of Milk (Milligrams per 100 ml.).		
						Yearly Average	Minimum	Maximum.
1944-45	I (spring calvers)	A	Jersey	12	20	0.25	0.23	0.27
		B	Jersey	12	20	0.25	0.21	0.34
		E	Friesian	4	20	0.20	0.16	0.24
		F	Friesian	4	20	0.20	0.16	0.28
1945-46	II (autumn calvers)	C	Friesian	4	20	0.20	0.15	0.24
		D	Friesian	4	20	0.18	0.14	0.22
		E	Jersey	4	20	0.27	0.21	0.33
		F	Jersey	4	20	0.25	0.19	0.29
1945-46	III	HP	Jersey	8	17	0.25	0.21	0.31
		LP	Jersey	8	17	0.27	0.21	0.33

the same order as those quoted for milks in other countries. Sherman(7) quotes the range of values within which the average for milk will probably be found as 19 mg. to 24 mg. per 100 g., and the United States Department of Agriculture(8) gives the average value as 0.17 mg. per 100 g. Daniell and Norris(14) quote the average values for fresh liquid whole milk as 0.177 mg. per 100 g. Holmes *et al*(10) quote 0.137 mg. per 100 ml., and Kon(12) states that mixed milk in England contains about 0.15 mg. per 100 ml. when cows are on pasture and about 0.10 mg. per 100 ml. during the stall feeding period. Holmes and Holmes(15) found values ranging from 0.113 mg. to 0.175 mg.



per 100 ml. for eighty samples taken during December, January, February, and March from cows on a diet of hay, wheat and grass silage, beet pulp, mangels, and ground grain. Stamberg and Theophilus(16) found values ranging from 0.156 mg. to 0.178 mg. per 100 ml. for some mixed milks, and Pearson *et al*(25) found the average for normal mixed milk to be 0.177 mg. per 100 ml.

#### EFFECT OF FEEDING CONDITIONS ON RIBOFLAVIN CONTENT

The supplementary feeding of meals to cows kept under normal New Zealand summer feeding conditions had no effect on the riboflavin content of milk of either Friesian or Jersey cows (Series I, Table II, and Fig. 2). There was a slight difference—viz., 0.02 mg. per 100 ml.—in favour of the groups not receiving meals for both the Friesian and Jersey milks in the Series II trials, and this difference persisted throughout most of the lactation. In view of the small number of cows in each group in Series II and the absence of an effect of meal feeding on riboflavin content in the Series I trials, the differences noted in the riboflavin content for the groups in the Series II trials cannot with confidence be attributed to the difference in feeding conditions. Whitnah *et al*(17), however, found a slight increase in riboflavin content of milk when the cows were turned on to pasture. Hand and Sharp(18) reported an average riboflavin content of 0.164 mg. per 100 ml. for commercial milk samples from indoor-fed cows and 0.197 mg. per 100 ml. for pasture-fed cows—i.e., the commercial summer milk contained 20 per cent. more riboflavin than the winter milk (see also Kramer *et al*(19))—and Houston *et al*(20) found a rise in the riboflavin level of commercial milk in England coincident with the change from winter to summer feeding conditions. Houston *et al*(1) followed the riboflavin content of two groups of Shorthorn cows, one of which was turned out to pasture thirty days after calving, while the other was kept indoors for a further ninety days. The line on their graph for riboflavin content for the milk from the outdoor group diverged from the line for the group kept indoors immediately after the change in feeding conditions, and the riboflavin content of the milk from this group remained higher than that for the indoor group throughout the period of indoor feeding of the latter group. During the subsequent period when both groups were at pasture, the riboflavin content of the milks were of approximately the same order. Theophilus and Stamberg(2) found that supplementing a ration of hay and concentrates with sunflower silage caused within two days a 36 per cent. increase in riboflavin content, and the riboflavin content remained high so long as the silage was fed. Zollikofer and Richard(21), on the other hand, found little variation, on the average, between the riboflavin content of milks when cows were fed on hay (0.138 mg. and 0.156 mg. per 100 ml.) and when they were fed on three varieties of silage (0.147 mg., 0.161 mg., and 0.155 mg. per 100 ml. respectively), and Hand and Sharp(18) found no change in riboflavin content when cows were changed from indoor feeding on molasses silage or phosphoric-acid silage to outdoor pasture feeding.

The milk from cows wintered on a high plane of nutrition (Series III) had a slightly lower yearly average riboflavin content than the milk from cows wintered on a low plane of nutrition. This yearly average difference is the result of differences occurring in the milks at the beginning and towards the end of the lactation period (see Fig. 2). It is not established whether this difference is due to the variation in feeding conditions or to the lower fat content of the milks from the HP group of cows.

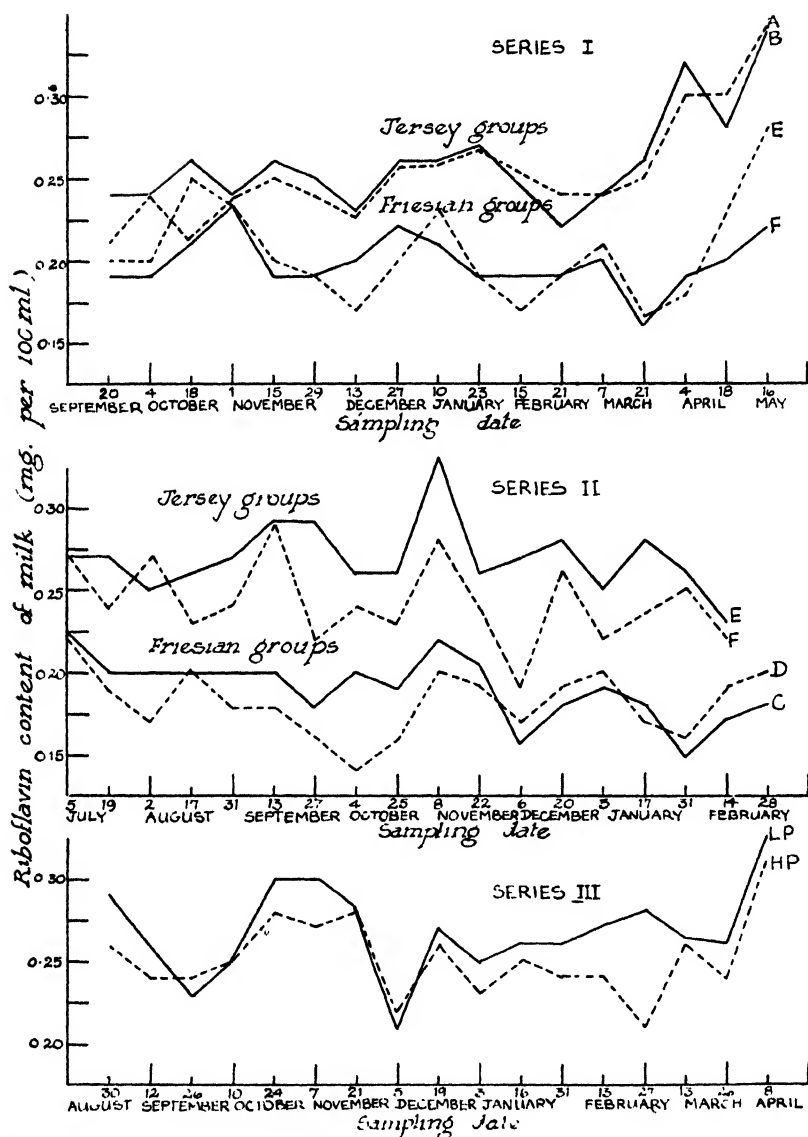


FIG. 2.—Riboflavin content of mixed milks from each group of cows in the three series of trials.

Series I and II .. — Cows fed on pasture.  
 .. - - - Cows fed on pasture plus meals.  
 Series III .. — Cows wintered on low plane of nutrition.  
 .. - - - Cows wintered on high plane of nutrition.

The results for riboflavin content did not in any instance fall to the value of 0.10 mg. per 100 ml. quoted by Kon(12) for cows in England during the stall feeding period, and only in one sample did they fall below the average value of 0.15 mg. per 100 ml. quoted by Kon for cows at pasture in England.

#### EFFECT OF STAGE OF LACTATION ON RIBOFLAVIN CONTENT

The variation in the riboflavin content with stage of lactation is not consistent in the three series of results. In Series I and Series III milks (Fig. 2) there was a definite rise in riboflavin content at the end of lactation which does not occur in Series II milks. This may be due to the balancing effect of any influence of feeding conditions on riboflavin content for Series III cows, whose lactations were completed in February. Apart from the end-of-lactation effect, there is no general trend of variation with stage of lactation deducible from the results. Whitnah *et al*(17) found little difference between the average riboflavin content of milks drawn during the first five months of lactation and those drawn during the second five months. Theophilus and Stamberg(2) found that colostrum samples taken within ten hours after calving contained 2.5 to 3.5 times as much riboflavin as normal milk, but after six to eight days after calving there was no further decrease in the riboflavin content. Houston *et al*(1) found high riboflavin values for the first and second colostrum, but report that the concentration declined rapidly to normal milk values; and Pearson *et al*(25) found that the riboflavin content of cows' milk attained the level for normal milk within less than a week after parturition.

#### EFFECT OF BREED OF COW ON RIBOFLAVIN CONTENT

The results for the Series I and Series II trials in Table I show a clearly defined difference in the yearly average riboflavin content of the milks from the different breeds of cows. In the Series I trials the yearly average riboflavin content for the Jersey group was 0.05 mg., and in the Series II trials 0.07 mg., per 100 ml. higher than for the corresponding Friesian groups. The yearly average fat tests of the milks were 5.07 and 3.48 for the Series I trials and 5.49 and 3.71 for the Series II trials respectively. The lines in Fig. 2 show that the differences persisted throughout the whole lactation, but in the Series I trial there was a greater increase in the difference with advance of lactation than with the Series II trial.

The yearly average values for riboflavin content of milk from the individual breeds of cows was fairly constant over the three series of results—viz., 0.25 mg. to 0.27 mg. per 100 ml. for the Jersey cows and 0.18 mg. to 0.20 mg. per 100 ml. for the Friesian cows—in spite of quite considerable variations in yearly average fat content of the milks from each breed of cow.

The difference found in riboflavin content of milks from the different breeds of cow is in line with the findings of other workers. Kon(12) states that the milk of cows of the Channel Islands' breeds contains more riboflavin than that of other cattle, and that the summer value may be up to 0.25 mg. per 100 ml. Whitnah *et al*(17) report the following values (in milligrams per 100 g.) as the average of about forty analyses of milks from each group: experimental Holsteins,  $0.143 \pm 0.053$ ; herd Holsteins,  $0.137 \pm 0.043$ ; Ayrshires,  $0.117 \pm 0.036$ ; Guernseys,  $0.153 \pm 0.048$ ; Jerseys,  $0.173 \pm 0.042$ .

Hand and Sharp(18) found that Holstein milk contained, on the average, 33 per cent. less riboflavin than Jersey milk, and reported higher values for milks from each breed of cow under pasture-feeding conditions, as shown in Table III.

TABLE III.—RIBOFLAVIN CONTENT OF MILKS FROM DIFFERENT BREEDS OF COWS  
(After Hand and Sharp(18) )

Breed of Cow.	Stall-fed.			Pasture-fed.		
	Number of Analyses.	Milligrams Riboflavin per 100 ml. Whole Milk.		Number of Analyses.	Milligrams Riboflavin per 100 ml. Whole Milk.	
		Range.	Average.		Range.	Average.
Jersey .. ..	25	0.155-0.342	0.218	24	0.190-0.340	0.237
Fuernesey ..	14	0.164-0.272	0.191	14	0.141-0.252	0.204
Brown Swiss ..	4	0.180-0.208	0.187	5	0.200-0.262	0.237
Ayrshire ..	9	0.126-0.208	0.169	10	0.157-0.238	0.197
Holstein ..	22	0.060-0.231	0.142	73	0.119-0.244	0.164
Whole herd ..	74	0.060-0.342	0.180	76	0.119-0.340	0.204

For comparison with these results the range of values for the two herds of cows in the present investigation are shown in Table IV.

TABLE IV.—RANGE OF VALUES FOR RIBOFLAVIN CONTENT OF JERSEY AND FRIESIAN MILKS IN THE THREE TRIALS

Breed of Cow.	Series.	Number of Cows.	Number of Analyses.	Minimum.	Maximum.	Yearly Average Riboflavin Content of Milk.
Jersey .. ..	I	24	40	0.21	0.34	0.250
	II	8	40	0.19	0.33	0.258
	III	16	34	0.21	0.33	0.260
All Jersey results	..	48	114	0.19	0.34	0.254
Friesian ..	I	4	40	0.16	0.28	0.200
	II	4	40	0.14	0.22	0.190
All Friesian results	..	8	80	0.14	0.28	0.194

The maximum values for the two breeds are thus of the same order as those recorded by Hand and Sharp, the minimum values being much higher than their minimum values, but whereas Hand and Sharp carried out their analyses on milks from individual cows (and it has been shown that the riboflavin content can vary widely with cow individuality), the results presented in this paper refer to mixed milks from groups of four to twelve cows.

#### SUMMARY OF DISCUSSION ON RIBOFLAVIN CONTENT OF MILK

The average values for riboflavin content of the New Zealand milks examined are within the upper range of values quoted for cow milks by overseas workers. Supplementary feeding of meals to cows kept under

summer feeding conditions had no effect on the riboflavin content of the milk. The small difference found for cows under winter feeding conditions (lower values for cows receiving meals) may not be significant, although it is in line with the findings of other workers that riboflavin content of milk is greater for cows on pasture alone than for cows on dry feeding alone. The consumption of meals by the cow may have reduced its appetite for pasture. There was no regular variation in riboflavin content with progress of lactation. Jersey milk had a higher riboflavin content than Friesian milk.

### NIACIN

The results are given in Table V and Fig. 3. The yearly average niacin content of the milks from the various groups of cows showed variations from 0.09 mg. to 0.12 mg. per 100 ml. of milk. There was no difference attributable to variations in the feeding conditions within each trial—i.e., supplementary feeding of meals (Series I and II) and conditions of wintering the cows (Series III)—or to difference in breed of cow or fat content of the milk. The milks from the autumn-calving cows (Series II) showed a general slightly higher level of niacin content than the milks from the spring-calving cows (Series I and III), but again the evidence is not adequate to establish that this difference was due to difference in season of lactation.

TABLE V.—YEARLY AVERAGE, MINIMUM AND MAXIMUM NIACIN CONTENTS OF MILKS FROM EACH GROUP OF COWS IN THE THREE SERIES OF TRIALS

Year.	Series.	Group.	Breed of Cow.	Number of Cows.	Number of Analyses.	Yearly Average Fat Test.	Niacin Content of Milk (Milligrams per 100 ml.).		
							Yearly Average.	Minimum.	Maximum.
1944-45	I (spring calvers)	A	Jersey	12	20	5.17	0.10	0.08	0.13
		B	Jersey	12	20	4.99	0.09	0.08	0.11
		E	Friesian	4	20	3.44	0.10	0.09	0.13
		F	Friesian	4	20	3.51	0.10	0.085	0.125
1945-46	II (autumn calvers)	C	Friesian	4	20	3.71	0.11	0.09	0.13
		D	Friesian	4	20	3.71	0.10	0.08	0.12
		E	Jersey	4	20	5.45	0.11	0.09	0.15
		F	Jersey	4	20	5.53	0.12	0.10	0.14
1945-46	III	HP	Jersey	8	17	5.28	0.096	0.08	0.10
		LP	Jersey	8	17	5.40	0.102	0.08	0.11

A fall in niacin content with progress of lactation occurred for all four groups in Series I, but there was no regular change with progress of lactation in either the Series II or the Series III trials. Pearson *et al*(25) found no significant difference in the nicotinic acid content of colostrum and of normal milk.

The average results for niacin content of the milks are in line with those reported for milks in overseas countries. The United States Department of Agriculture(8) quotes 0.1 mg. per 100 g. as the average niacin content, Holmes *et al*(10) found 0.11 mg.  $\pm$  0.01 mg. per 100 ml. for late-summer milk from a mixed herd of cows in United States, Teply *et al*(22) found

0.08 mg. per 100 g., and Bailey *et al*(24) found an average value of 0.146 mg. per 100 ml. for the milks of six Ayrshire cows. McVicar and Berryman(23), in a review of the literature, place the probable average value at 0.1 g. per 100 g., although values ranging from 0.05 mg. to 0.82 mg. per 100 g. have been quoted by various other workers (some of this reported variation appears to be due to errors of the methods used for estimation).

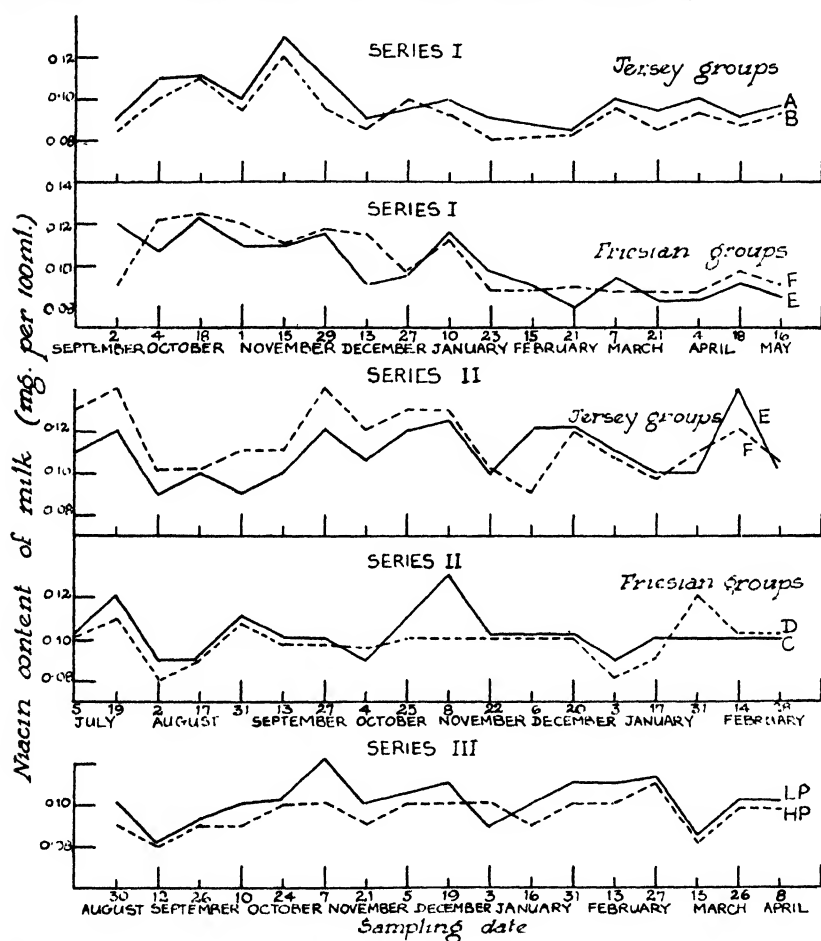


FIG. 3.—Niacin content of mixed milks from each group of cows in the three series of trials.

- |                    |      |   |
|--------------------|------|---|
| Series I and II .. | ——   | Cows fed on pasture.                      |
|                    | ---- | Cows fed on pasture plus meals.           |
| Series III ..      | ——   | Cows wintered on low plane of nutrition.  |
|                    | ---- | Cows wintered on high plane of nutrition. |

#### ACKNOWLEDGMENTS

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## SOIL DISINFECTION

### VI. AN INJECTOR FOR APPLYING SMALL DOSAGES OF VOLATILE FUMIGANTS TO SOILS

By H. JACKS, Plant Pathologist, and L. WRIGHT, Technician, Plant Diseases Division, Plant Research Bureau, Department of Scientific and Industrial Research

[Received for publication, 20th September, 1946]

#### Summary

An instrument for injection of controlled dosages of volatile liquids into the soil has been designed. A detailed description of the instrument is given and its performance in practice discussed.

#### INTRODUCTION

VOLATILE liquids such as chloropicrin, Shell DD, carbon disulphide, methyl bromide, ethylene dichloride, and mixtures of these are used for soil disinfection. They are injected into the soil, and it is essential that dosages for each injection be accurately measured and that escape of fumigant other than in the soil be avoided to ensure safe usage.

In experimental work a measured amount of fumigant was applied from a graduated burette into holes dibbled in the soil. This method was, however, too tedious for practical purposes, and several instruments subsequently tested proved unsatisfactory. The requirements essential for effective application are enumerated below :—

- (1) Continuous operation, whereby liquid is drawn from a reservoir.
- (2) Accuracy of measurement of small dosages (2 ml. to 5 ml.) in successive injections.
- (3) Resistance to corrosive action of fumigants, particularly on the materials used for washers.
- (4) Tightness to prevent gas escape.
- (5) Ease of dismantling for cleaning purposes.

The development of an injector possessing these essential features was found to be necessary. It was found that brass was sufficiently resistant to corrosion and was easily machined. Several models were made. The principle of using an air-cushion to expel the dosage required was found to be unsatisfactory, as the air tended to flow past the liquid. Instruments in which the fumigant was required to flow through a spring-loaded valve into a measuring-chamber were unsatisfactory owing to the difficulty of securing the correct loading for accurate operation of the valve.



FIG. 1.—The assembled soil injector.

The instrument described below includes logical improvements. It is made of very few parts, contains only one easily replaceable washer, and of the models so far tested has been found to give the most satisfactory results. Several lubricants were tested, and of these, paraffin-oil alone did not cause gumminess.

#### DESCRIPTION OF INSTRUMENT

This instrument is constructed as a hand-operated pump and includes a mechanism for the exact regulation of dosage rates. It consists of three parts: (1) the reservoir and cylinder; (2) the piston assembly; (3) the delivery tube and valve assembly.

(1) The reservoir (A) is gas-tight and can hold over 1 pint (600 ml.) of fumigant. It is filled through an opening closed by cap (B), which also serves as air inlet. A cylinder (C) internally machined to fit the hand-operated piston (D) runs through the reservoir. Holes drilled in the cylinder wall (G) connect the reservoir and the cylinder and are uncovered when the piston is fully raised.

(2) The piston assembly comprises the piston (D), the sleeve (E) forming the upper and the adjustable collar (F) the lower limit for piston movements. The piston-rod is held in place by a retaining-nut and is provided with a hand-grip for manipulation.



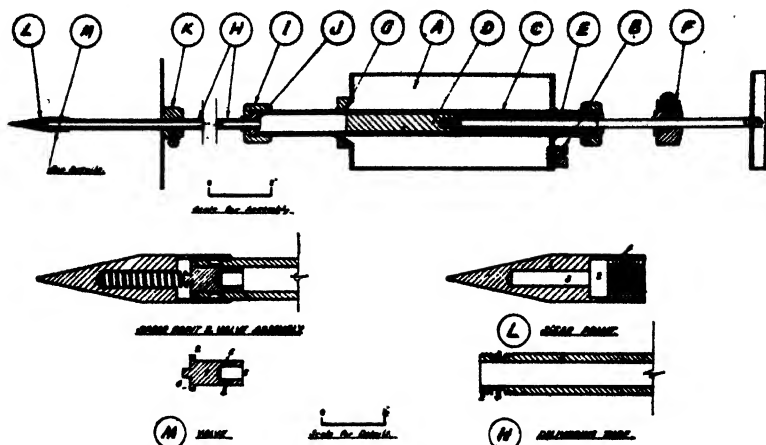


FIG. 2.—Illustrating construction of the soil injector.

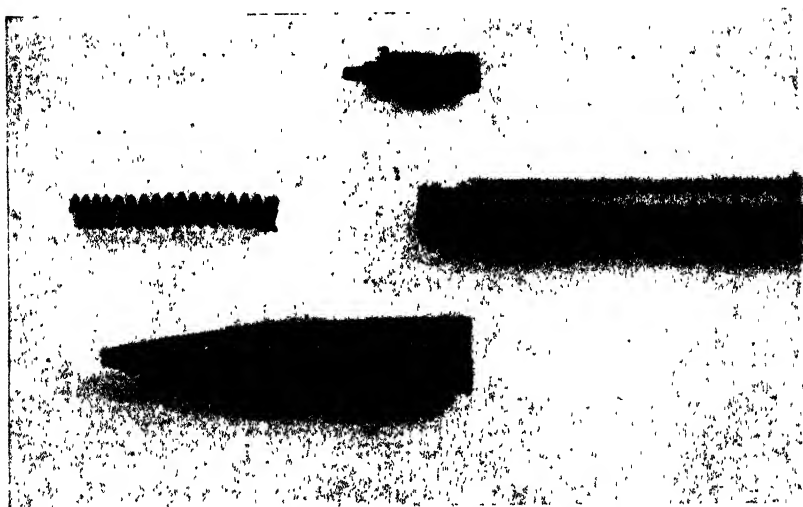


FIG. 3.—Illustrating spearpoint and valve assembly.



FIG. 4.—Showing the three main parts of the injector.

(3) The delivery tube (H) fits into the lower end of the cylinder and is held in place by the retaining-nut (I). A replaceable packing washer (J) prevents leakage at this point. Depth of injection is regulated by the soil-depth gauge (K), which is adjustable along the delivery tube and can be locked in the desired position by the clamping-screw. The lower end of the delivery tube (H1) is threaded (H2) to fit into the spearpoint (L2), and a groove (H3) cut below the threads is provided with a small hole (H4) corresponding with an outlet in the spearpoint (L4).

The spearpoint (L) consists of body (L1), a recess (L2) drilled and tapped to fit on to delivery tube (H2), with clearance beyond the movement of valve (M2), a spring (L3), and (L4) a hole corresponding with the position of opening in delivery tube (H4). The spearpoint contains the valve assembly (M).

The valve (M) is machined to slide into the lower end of delivery tube, and comprises the body (M1), a shoulder (M2) limiting up and down movement, an  $\frac{1}{8}$ -in.-diameter hole drilled to a depth of  $\frac{1}{8}$  in. (M3), a groove surrounding the body (M4), (M5) a smaller hole connecting (M3) and (M4), and a nipple (M6) for location of spring.

#### OPERATION

Care should be taken to ensure that the liquid is free of impurities when filling the reservoir. After filling the reservoir (A), the piston (D) is withdrawn to its upper limit. The holes (G) being uncovered, the fumigant flows into the lower end of the cylinder (C) and the delivery tube (H). The spring-loaded valve (M) is then in its normal position and prevents leakage of liquid through the spearhead (L). A downward movement of the piston closes the reservoir outlet holes, moves the valve downward, and forces the liquid through the respective injection holes in the valve (M3), in delivery tube (H4), and in the spearhead (L4). When withdrawing the piston, the valve is closed by the spring and a partial vacuum in the cylinder is filled by liquid when holes (G) are uncovered by the upward stroke of the piston. Adjustment of the collar (F) controls the dosage rate from 1 ml. to 10 ml. per stroke. The depth of injection is determined by position of (K).

After operation, the instrument is thoroughly washed out with kerosene or petrol and lubricated with paraffin-oil. Care should be taken to fill the space between the sleeve (E) and the cylinder (C) with oil to ensure continuous lubrication of the piston.

#### DISCUSSION

The above instrument was used for injection of highly volatile fumigants into the soil. No difficulty was encountered in filling the reservoir, nor was there obvious escape of fumigant. It was found that quantities to within 98 per cent. of the amount required could be applied effectively. Although satisfactory in soils of good tilth, it is probable that with rough usage a more sturdy spike might be desirable. For this purpose a steel tube with a brass lining would probably be suitable. The above injector was, however, used in lawns in holes previously prepared by insertion of a steel rod.

Leakages sometimes occur through the lodgment of soil particles under the valve seat, but these are easily removed by a few strokes of the piston. After prolonged use, leakages sometimes develop at the delivery-tube connection. These can easily be avoided by the frequent replacement of the fibre washer. For convenience of the operator an extension of the handle is desirable to reduce the need for stooping. It may also be found that a larger container, by reducing the frequency of refilling, would add to the efficiency of the injector.

## A NOTE ON CRINKLE IN NEW ZEALAND APPLES

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[Received for publication, 30th July, 1946]

### Summary

Crinkle, a physiological disorder of apples, appeared in New Zealand during the summer of 1945-46. Affected fruit show external depressions and a shallow layer of necrotic tissue in the flesh. Similar trouble has been reported from Australia, U.S.A., and England. High temperatures aggravated by drought appear to cause the disease.

AN unusual physiological disorder of apples appeared on a small scale in several parts of New Zealand during the 1945-46 season. This particular trouble has not been recorded previously in New Zealand.

Symptoms are briefly as follows. At maturity the fruit shows external distortion, generally at the calyx end. This may take the form of one small depression, a series of irregular depressions, or a continuous broad groove surrounding the calyx. In a few cases the skin above the depressed area appears dull brown, but more commonly it remains normal. The depressed areas are soft and spongy to the touch. Immediately beneath each depression lies a shallow zone of light-brown necrotic tissue. In typical specimens this necrotic tissue is spongy and split up by numerous large cavities (Fig. 1), but seldom extends more than 10 mm. into the flesh. The remainder of the apple is normal in both appearance and taste.

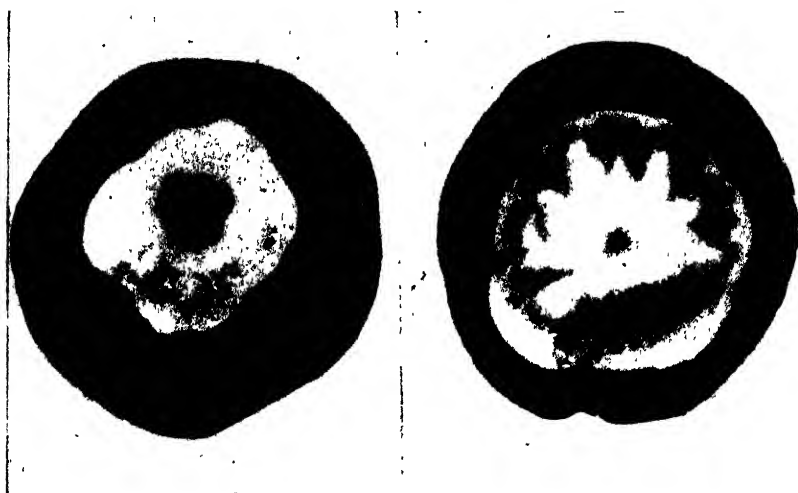
Apples showing these symptoms were recorded from Auckland, Hawke's Bay, and Nelson, the varieties affected being Winesap, Rome Beauty, and Sturmer respectively. Only a small number of apples were affected in each of the cases reported and the losses caused were negligible.

Apple disorders apparently identical with the condition detailed above have been reported from several countries. McAlpine (1901) recorded the occurrence of distorted apples in Australia. He named the trouble "crinkle" because the surface of the fruit was frequently thrown into rough folds, giving it an uneven, crinkled appearance. In a later publication McAlpine (1912) stated that in his opinion crinkle was a "confluent form" of bitter-pit. Brooks and Fisher (1918) reported a condition termed "hollow apple" in Washington. This occurred almost exclusively on apples exposed to direct sunlight and was most severe on trees bearing a light crop. Roberts (1919) found crinkle to be widespread in Wisconsin after a year of extreme drought.

He could find no relation between the position of fruit on the tree and the incidence of crinkle. Brooks and Fisher (1926) recorded the appearance of symptoms similar to crinkle on two occasions in the northern States of the United States of America. Their observations suggested that periods of high temperature following relatively cool weather were the primary cause, with a shortage of soil moisture as an aggravating factor. Four years later Fisher, Harley, and Brooks (1930) produced crinkle on a small number of shaded apples by applying artificial heat to the growing fruit.

Ware (1932) noted injury of the crinkle type on a small number of apples from the south of England. He also attributed the injury to high temperature. Carne and Martin (1934) found crinkle to occur quite commonly in Tasmania and the southern half of Australia. They claimed that water-core was an essential step in the development of crinkle, and that high temperatures were the primary cause. Under Australian conditions they found it possible to predict with reasonable accuracy that crinkle would appear in susceptible varieties ten to fourteen days after any marked heat wave in January or February.

Descriptions and illustrations given by these authors leave little doubt that all were dealing with forms of the one disease. Some of them attempted to isolate a pathogen from crinkle-affected apples without success, and all agree that the trouble is a physiological one. There is agreement on one



[Photo by L. H. Wright]

FIG. 1.—Rome Beauty apples showing symptoms of slight and severe crinkle.

other point—namely, the association of an unusual period of high temperature with the development of crinkle. The work of Fisher *et al.* supports these observations, and it appears reasonably certain that high temperature is the primary cause. Damage occurs after a period of weather hotter than is usual for the particular district, irrespective of the extreme maximum reached. Thus in the United States of America crinkle was found after shade temperatures of 107° F. and 110° F., which were 19° F. and 22° F. respectively above the mean of the locality affected. In England a few apples were affected when the maximum temperature reached 89° F. in a district where the mean was 60.9° F. Opinions differ considerably as to the effect of direct sunlight on the production of crinkle. Specimens found in New Zealand were not examined while on the trees, and there is no information available as to their relative exposure to light.

In the summer of 1945-46 New Zealand experienced a severe and widespread drought, with shade temperatures for January and February above normal. In Hastings, where symptoms were most pronounced, there was a marked temperature peak early in February. In Auckland there was a less pronounced peak at about the same period, and in Nelson a sudden rise took place early in January. Relevant figures are set out in the table below. The peak temperatures shown were the highest reached at the three recording stations during that summer.

TABLE I.—MAXIMUM DAILY SHADE TEMPERATURES, IN DEGREES FAHRENHEIT

Auckland.		Hastings.		Appleby (Nelson).	
Date.	Maximum Shade Temperature.	Date.	Maximum Shade Temperature.	Date.	Maximum Shade Temperature.
1946		1946		1946	
January 28 ..	68.6	February 4	94.0	January 9	62.3
January 29 ..	70.0	February 5	86.0	January 10	71.6
January 30 ..	72.5	February 6	81.0	January 11	77.3
January 31 ..	74.6	February 7	83.0	January 12	86.2
February 1 ..	75.3	February 8	86.0	January 13	83.6
February 2 ..	78.6	February 9	97.5	January 14	71.1
February 3 ..	79.6	February 10	71.0		
February 4 ..	82.6				
February 5 ..	75.2				
February 6 ..	72.0				
Mean daily maximum for January, 1946	72.3		80.6		72.8
Mean daily maximum for February, 1946	75.5		81.7		72.8
Average mean over period of years—					
January ..	72.6		77.0		71.6
February ..	73.0		76.0		71.0

At the close of this unusual season, crinkle appeared in three widely separated places, suggesting that, in New Zealand also, high temperature aggravated by drought caused the disease.

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## OBSERVATIONS ON SEEDLING FEIJOAS (*FEIJOA SELLOWIANA* BERG.) AT MOUNT ALBERT, AUCKLAND

By H. M. MOUAT, Pomologist, Plant Diseases Division, Department of Scientific and Industrial Research

[Received for publication, 16th August, 1946]

### Summary

An account is given of the growth and fruiting of seedling trees of *Feijoa sellowiana* Berg. at Mount Albert, Auckland.

Observations showed that plants varied considerably in type, and in the shape, size and quantity of fruit produced.

It is recommended that only trees worked with selected scion varieties be used for future plantings.

*Feijoa sellowiana* Berg., a native of the sub-tropical regions of South America, is a fairly hardy fruiting shrub of the family Myrtaceæ. Small commercial plantings have been made recently in the warmer parts of New Zealand. Since some of these consisted of seedlings and not of named varieties, it was desirable that information be obtained regarding the performance of such trees. A trial planting of seedling trees, set out as a hedge, was therefore made at the research station of the Plant Diseases Division, and the present paper records observations on these trees.

Seedlings raised in 1939 from a large-fruited type (variety unknown) were carefully selected for uniformity and grown in the nursery for two years. Sixty-eight trees were set out in 1941 in a single row, the distance between trees being 2 ft. These were left unpruned until 1945, when records of growth, fruit type, and yield were taken. Trees were cut back in the winter of 1945 to form a hedge, and the heavy pruning severely restricted fruit production in the following year.

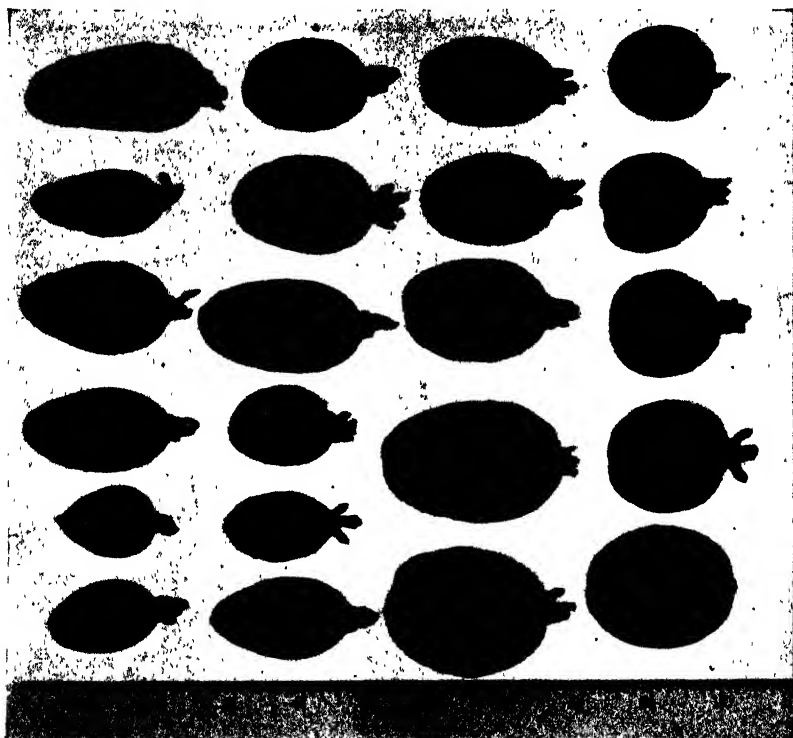
**Growth Type.**—It was found that, in spite of the initial selection of seedlings, there was a considerable variation in plant type. While the majority of trees were of an upright, strongly-growing bushy habit, others were of a more spreading open type. A few formed less vigorous, compact bushes with smaller leaves.

**Fruit Yield.**—A few bushes fruited in 1944, but no records were taken. Many fruited in 1945. Table I shows the crop harvested in April of that year. It will be seen that twenty-seven of the sixty-eight trees carried no fruit and twenty-three produced less than 1 lb. each. The crop produced by individual trees varied from a few ounces to almost 6 lb. per tree. Sixty per cent. of the total crop of approximately 50 lb. was produced by seven trees, or 10 per cent. of the total number. The production figures for one year, though not a reliable index of productivity over a number of years, do show the great variability of seedlings. Though only a small quantity of fruit was borne in 1946 as a result of the severe pruning, crop yields showed the same extreme variability as in the previous season.

**Fruit Type.**—The variation in size and shape of fruit from different trees is shown in Fig. 1. Fruit ranged from 3.5 cm. to 6.7 cm. in length, the majority being from 3.8 cm. to 4.5 cm. (Table II). The shape was most commonly broad oval, but varied from round to long oval and pyriform. Fruit was dark green in colour with a slight grey bloom, that from some trees having also a dull-red blush on one cheek. The surface varied from smooth to very rough. Fruits also varied in relative amounts of pulp and flesh, colour of flesh (white to pale yellow), and flavour.

## DISCUSSION

The trial has shown the great variability that exists in seedling plants of *Feijoa sellowiana* Berg. Differences in type of growth and tree size markedly greater than those found in the above trial were seen by the author in a commercial plantation of seedlings. For successful commercial fruit production it is essential that all trees in a grove should bear good crops regularly and that fruit should be uniform and of a size and type desired by the market. From the above observations it would appear that seedling trees of feijoa would not fulfil these conditions. Even if total



[Photo by R. Hughes.]

FIG. 1.—Variation in size and shape of fruit from seedlings of *F. sellowiana* Berg. (About  $\frac{2}{3}$  natural size.)

production of low-yielding trees increased in future years it is probable that an inherent variability would persist. Only about 25 per cent. of the trees in the trial bore fruit of good size. It is unlikely that increasing age will bring about increased size in small fruited strains. On the contrary, judging from the behaviour of other fruits, the reverse is more likely to occur.

As the feijoa is a recent introduction, consumers have not had time to show a preference for a particular type of fruit. However, fruit tapered toward the ends, or with a very irregular surface, would probably not be popular, since there would be more waste with these than with the smoother oval and round types.

It is considered that growers, when making new plantings of feijoas, should plant only trees worked with a scion variety known to produce large crops of fruit of good size and type. An additional argument in favour of growing a scion variety of known performance is the discovery by Doak (correspondence, 1946) that there is a wide variation in ascorbic acid (vitamin C) content of fruit from seedling feijoas. A sample of Coolidgei, a named variety of *F. sellowiana*, had a very high vitamin C content (116 mg. per 100 g.), more than three times as much as that of a small fruited seedling growing in the same garden. Considering the dietetic importance of this vitamin it is desirable that varieties known to have fruit with a high content should be grown rather than seedlings, for, although Doak found no correlation between fruit size and vitamin C content, it is apparent that fruit from some seedlings contains a relatively low percentage.

TABLE I.—RECORD OF CROP EX TREES FRUITING 1945

Weight of Fruit per Tree.				Number of Trees.
lb.				
No crop	..	..	..	27
0.5 and under	..	..	..	15
0.6 - 1.0	..	..	..	8
1.1 - 1.5	..	..	..	4
1.6 - 2.0	..	..	..	5
2.1 - 2.5	..	..	..	1
2.6 - 3.0	..	..	..	2
3.1 - 3.5	..	..	..	1
3.6 - 4.0	..	..	..	0
4.1 - 4.5	..	..	..	3
4.6 - 5.0	..	..	..	1
5.1 - 5.5	..	..	..	0
5.6 - 6.0	..	..	..	1

TABLE II.—VARIATION IN SIZE OF FRUIT PRODUCED, 1945

Length of Fruit.				Number of Trees.
cm.				
3.5	..	..	..	1
3.8	..	..	..	9
4.1	..	..	..	8
4.5	..	..	..	12
4.8	..	..	..	2
5.0	..	..	..	6
5.7	..	..	..	2
6.7	..	..	..	1



## FLUE-CURED TOBACCO

### II. DEVELOPMENT OF SUGARS IN LEAF DURING CURING

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*(Continued from Vol. 26 (Sec. A), page 171)*

*[Received for publication, 23rd September, 1946]*

#### Summary

During flue-curing of tobacco-leaf there is a rapid production of reducing sugars, mainly glucose, together with small amounts of sucrose by hydrolysis of starch and possibly other carbohydrates. After the colouring stage, change in the sugar content of the leaf is small. For the whole leaf up to nearly 26 per cent. of total sugars on the dry basis, of which 20 per cent. was glucose, have been found.

The blade of the leaf contains more sugar than the midrib. In one experiment 27.7 per cent. of total sugars was found in the blade and 20.6 per cent. in the midrib.

Loss of dry matter during curing can be closely accounted for by assuming that respiration is dependent on oxidation of hexoses. Participation of other compounds in these losses is not precluded, however.

#### INTRODUCTION

THE chief distinction between flue-cured tobacco, used largely for manufacture of cigarettes, and air-dried tobacco, used for cigars and certain other types of manufactured tobacco, is the relatively high content of the reducing sugars, glucose and lævulose, and of sucrose, compared with the almost total absence of sugars in the air-dried types of leaf. Although there are data in the literature to substantiate this, there is a definite lack of information on the course of development of these sugars in the leaf during the flue-curing procedure.

In 1941 Roberts(1) carried out an investigation on the changes in respiration rate of the leaf at different stages, but no measurements were made before twenty-four hours of curing. It was concluded that in the earlier stages respiration resulted in the consumption of carbohydrates and organic acids, but that later, although these compounds were still accounting for the greater part of the respiration, protein was also being metabolized. This effect had appeared after forty hours of curing. It was suggested, too, that a considerable period of heating at 120° F. was required to inactivate the enzymes so that discoloration of the leaf would not occur if moisture were absorbed. This would correspond to the fixing stage of curing. In the same year the Bureau of Plant Industry of the U.S. Department of Agriculture reported(2) that experimental work on flue-curing had been carried out. Beyond a short report, the authors are not aware of any published statement of data or final conclusions from these trials. The major chemical change reported was the transformation of carbohydrates, this consisting largely of hydrolysis of starch to sucrose and a mixture of dextrose and lævulose; the sucrose was afterwards converted into reducing sugars. No numerical data were given to indicate the magnitude or precise direction of the changes at different stages of curing.

The study of curing most pertinent to the authors' work is that of Ward(3), who followed the changes in carbohydrate contents of Canadian leaf. Ward showed that the glucose, lævulose, and sucrose contents increased to maxima, and then decreased in the later stages of curing. Starch, on the other hand, progressively decreased with length of time of the leaf in the kiln. As much as 34 per cent. of total sugars was found in the dry matter.

In the experiments to be described in this report a great development of sugars was found during curing of the leaf.

#### EXPERIMENTAL

Three experiments were carried out in the 1943 season, commencing on 18th February, 4th March, and 11th March. After removal from the kiln the sample material was rapidly dried by hot air. It is possible that this procedure increased the amount of sugars found on analysis, especially in the early stages of curing when the leaf still contained a high percentage of water, but, judging by the low sugar content of the leaf-blade in the first sample of experiment III, this effect was probably small. The sample material was analysed approximately six months after completion of the curing trials. In experiments I and II whole leaf was analysed, but in III separations of blade and midrib were made at the several sampling periods. Further details in respect of the crop have been reported previously(4). The method of extraction of the sugars has been given by Blick(5). Glucose was estimated by hypo-iodite oxidation; the difference between the total reducing sugars and glucose was called lævulose. Proof that only these two reducing sugars were present is not available. Sucrose content was obtained from the total reducing sugars before and after hydrolysis of the cleared solution with hydrochloric acid. No other carbohydrates have been estimated.

#### RESULTS

The data for the sugar contents in the first two experiments, together with certain other experimental data, are set out in Table I. During the colouring period, when the temperature is low, and the relative humidity and moisture content of the leaf are high, increase in amounts of sugars in the leaf is rapid. Within a relatively few hours from entering the kiln the sugars, more especially glucose, begin to form. A certain amount of lævulose also appears, providing about one-fifth of the total sugars in experiment I, but only about one-tenth in experiment II. Sucrose is higher in experiment I than in experiment II, and, except in the early stages of the first experiment, forms only a small proportion of the total sugars. Once the temperature has been raised sufficiently high in the fixing stage (to about 120–130° F.) to cause rapid drying and to kill the leaf, no further marked changes in sugar content are to be found. The final temperatures, too, have not been sufficient to cause decomposition of the sugars. Moreover, in these two experiments there is no evidence of maxima in the sugar contents as was reported by Ward(3).

TABLE I.—DEVELOPMENT OF SUGARS DURING FLUE-CURING OF TOBACCO-LEAF  
Sugar content expressed as percentage on dry-matter basis

Experiment No.	Number of Hours in Kiln.	Glucose, per Cent.	Fructose, per Cent.	Sucrose, per Cent.	Total Sugars, per Cent.	Moisture in Leaf, per Cent.	Loss of Dry Matter, per Cent.	Relative Humidity, per Cent.	Dry-bulb Temperature (°F.).
I	0	6.20	0.54	0.00	6.74	85.2	..	..	..
	6	11.73	1.02	5.08	17.83	83.1	..	84	87
	12	15.27	2.08	3.29	20.64	83.2	..	84	86
	18	15.53	4.78	2.08	22.39	82.7	..	87	85
	24	15.01	4.51	2.42	21.94	83.4	4.4	92	88
	30	16.37	4.16	2.09	22.62	81.0	3.7	88	88
	36	15.61	3.71	1.15	20.47	82.1	2.0	88	88
	48	14.99	4.45	0.00	19.44	81.7	13.6	81	93
	60	13.44	2.88	1.82	18.14	83.6	11.1	92	92
	72	16.48	6.94	0.00	23.42	80.9	11.0	45	120
	84	14.52	3.38	0.62	18.52	86.5	11.8	21	132
	108	13.46	4.81	1.01	19.28	4.6	11.7	n.d.	155
	132	16.95	5.59	0.99	23.53	10.2	9.0	n.d.	170
II	0	7.33	0.67	0.00	8.00	82.6	..	..	..
	6	7.08	2.43	1.09	10.54	80.6	0.5	72	87
	12	10.24	0.00	1.09	11.33	79.9	3.8	78	81
	18	15.26	2.26	2.66	20.18	81.2	12.7	79	82
	24	13.21	0.79	0.88	14.88	81.7	17.3	70	91
	30	13.08	0.75	0.00	13.83	78.6	12.2	79	85
	36	14.63	1.39	0.94	16.96	78.9	13.7	79	83
	48	16.72	2.39	0.86	19.97	74.4	8.3	76	87
	60	19.39	4.64	0.87	24.90	67.0	9.2	61	106
	72	19.49	3.02	1.33	23.84	47.8	9.9	37	122
	84	11.41?	0.72?	2.50	14.63?	40.7	13.7	24	124
	108	20.02	4.04	1.62	25.68	22.5	8.5	n.d.	160

NOTE.—Average weights of leaves were—Experiment I, fresh 39.4 g., dry 5.95 g.: experiment II, fresh 36.75 g., dry 6.36 g.

In the third experiment the midribs were removed from leaves on taking them from the kiln. Blade and midrib were then rapidly dried before grinding preparatory to analysis. The leaves used in this experiment, from near the top of the plants, were rather small, weighing approximately 20 g. when fresh and having 19.1 per cent. of dry matter. Table II gives the analytical data, the curing conditions having been already reported(4).

In the blades the initial sugar contents are low, totalling only 3.21 per cent., but within twelve hours the glucose and total sugars have increased fivefold and levulose and sucrose have also appeared. By the end of the colouring period (sixty hours) the transformations into glucose have almost been completed. Fructose continues to increase, but sucrose has fallen to low values at this time. The midribs show a relatively high content of sugars, especially of glucose. Both glucose and fructose show appreciable increases during curing, but neither reach the same level that they do in the blades. Sucrose varies irregularly, but tends to be higher in the midribs than in the blades. The highest figure for total sugars was 27.7 per cent. in the blades and 20.6 per cent. in midribs.

TABLE II.—DEVELOPMENT OF SUGARS IN BLADE AND MIDRIB OF TOBACCO-LEAF DURING FLUE-CURING

Sugar content expressed as percentage on dry matter basis

Part of Leaf.	Hours in Kiln.	Glucose, per Cent.	Fructose, per Cent.	Sucrose, per Cent.	Total Sugars, per Cent.	Moisture in Sample, per Cent.	Loss of Dry Matter, per Cent.
Blade .. ..	0	2.24	0.00	0.97	3.21	77.2	..
	12	11.95	2.33	3.32	17.60	72.4	0.0
	36	19.27	2.63	1.35	23.25	69.4	4.7
	60	18.94	5.73	0.00	24.67	65.5	16.0
	84	19.30	6.29	0.43	26.02	36.0	15.4
	108	18.38	8.60	0.74	27.72	10.4	15.5
	132	20.30	6.10	0.61	27.01	0.0	11.1
Midrib .. ..	0	8.46	0.43	1.65	10.54	88.3	..
	12	8.43	1.55	2.88	12.86	81.7	0.0
	36	12.30	1.04	0.63	13.97	79.7	12.6
	60	13.90	3.06	0.00	16.96	78.1	11.5
	84	14.13	3.24	3.24	20.61	73.6	11.6
	108	11.51	4.51	2.09	18.11	52.9	13.7
	132	12.78	3.45	1.25	17.48	44.2	13.7

## DISCUSSION

Examination of Ward's results(3) shows that lævulose reached maximum values usually forty-eight to sixty hours after entering the kiln, while dextrose exhibited maxima at about sixty hours. Sucrose, in six cases out of eight, allowing for probable experimental error in the determinations, continued to increase until the end of the curing operation. Total sugars tended to increase until the end of the fixing period, at approximately 130° F. It is to be noted, too, that Ward's curing conditions called for more rapid raising of the temperature than was the case in the authors' experiments. Setting out Ward's results in graphical form, as has been done in Fig. 1 for one of his trials (second priming, 1937), indicates that the glucose, lævulose, and sucrose contents increase linearly with increase of time in the kiln, up to approximately sixty hours. On the other hand, starch, and hemi-celluloses (not shown in Fig. 1), decrease linearly with time. In certain cases there is departure from linearity for all these constituents, but it is not very great.

The data plotted in Fig. 1 are from the most consistent data—i.e., those least affected by apparently aberrant figures. In this graph the lines for the three sugars are almost parallel, with the lævulose and sucrose percentages almost equal at a given time. For other experiments sucrose is sometimes present in greater amount than lævulose, especially in the earlier stages of curing; in all cases, however, sucrose and lævulose are approximately equal in amount. The dextrose curve for some of Ward's experiments was not parallel to those of the other sugars, but indicated that this sugar increased at a greater rate than the lævulose and sucrose. How far the experimental conditions of Ward's experiments contribute to these linear variations it is not possible to say. It is quite clear, however, that the variation of concentration of sugars with time is quite different in these Canadian experiments from that of the New Zealand ones, typified by the graphs in Fig. 2.

These indicate that a rapid increase in glucose and total sugar content occurred during the first forty-eight hours (colouring period), with only slight increases during fixing (up to about eighty hours) and drying of the blade and the midrib. There is no definite evidence that the glucose or total sugars contents pass through maxima, when these are expressed on the dry matter of the sample at the stated times of curing. If the sugars are expressed in terms of the original dry-matter content of the leaves (blades or midribs) using for the calculations data for loss of dry matter given in Tables I and II, the curves showing the sugar content at various stages are much flatter than

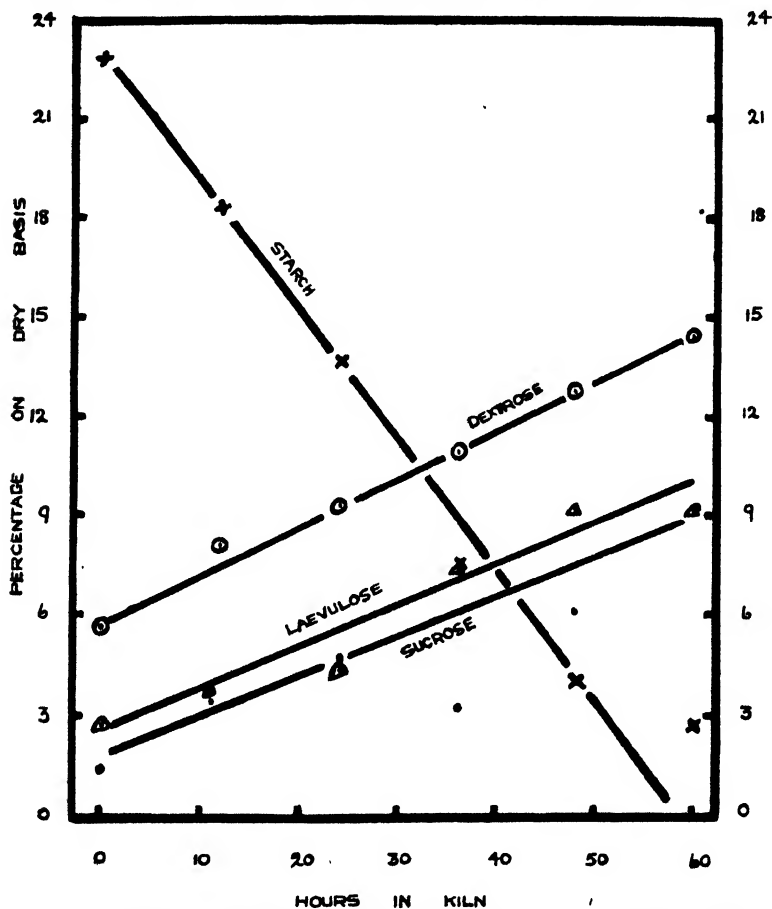


FIG. 1.—Variation of sugar and starch contents during curing (Ward, 1942).

those of Fig. 2, but they still do not show any maxima. The same holds true for the data of Table I. It is clear also that the concentration of sugars at any chosen time must be the resultant between that formed by hydrolysis of more complex carbohydrates and that lost by respiration. The figures for sugar content obtained in the present trials are not as high as those of Ward, most probably because the leaf used was taken from much higher up the plant than was Ward's. Unpublished data obtained from successive harvests of leaves show that there are large differences in the sugar contents of cured leaf from varying positions on the plant, relatively low figures being found for leaf from near the top of the plant.

In the first paper of this series(4) attention was drawn to the large loss of dry matter from the leaf during curing. This was of the order of 14 per cent. on the average, and was shown to occur almost wholly during the colouring or early fixing stages. Respiration with consumption of carbohydrates and possibly other compounds is responsible for this loss. Work in Australia(6) has demonstrated that large amounts of carbon dioxide are produced during curing. It would appear that about 360 c.c. of carbon dioxide per pound of leaf per hour are formed. This evolution of gas corresponds to about 80 c.c. per 100 g. per hour. Now, if the time of colouring is thirty hours (a conservative estimate), then 2,400 c.c. of carbon dioxide will be produced per 100 g. of leaf. This volume of gas will weigh 4.7 g. A hexose on oxidation will give 1.5 g. of carbon dioxide per gram; therefore, the above weight of gas corresponds to the consumption of 3.2 g. of sugar (glucose or lævulose).

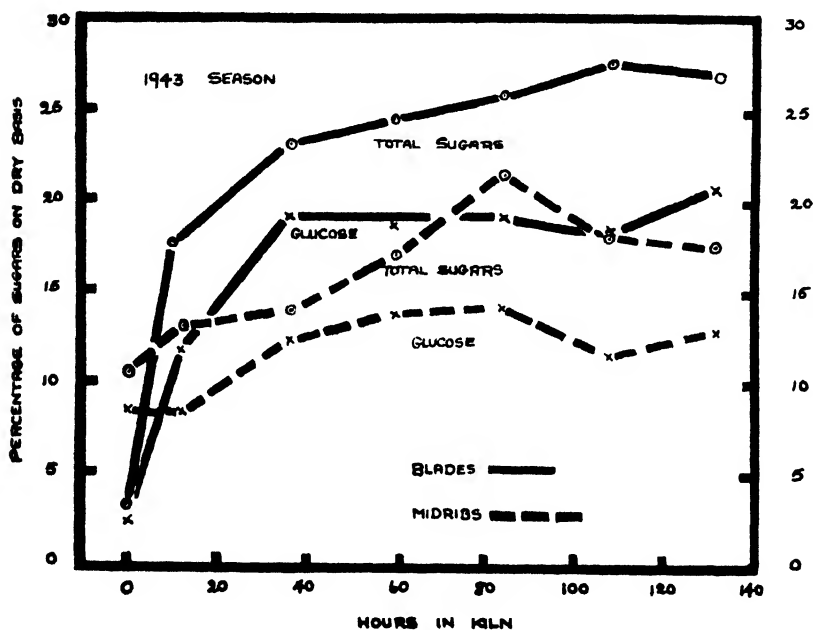


FIG. 2.—Variation of reducing sugars and total sugars of blade and midrib during curing.

Data(7) in the publication just quoted show a loss of 13 per cent. of dry matter from leaf of an original dry-matter content of 25 per cent. This shows that 3.25 g. of dry matter disappeared during curing. Taking the Nelson data of 14 per cent. loss on leaf with 20 per cent. dry matter, a loss of 2.8 g. is obtained. Both of these losses correspond closely to the loss calculated on the assumption that hexoses (or a hexose) provide all the substrate for respiration. This does not mean that other carbohydrates, or acids, or nitrogenous compounds are precluded from contributing to the loss of dry matter, but to what extent these take part does not appear to be known. With air-dried leaf the respiration processes are so extensive that practically no sugars remain on completion of curing, and a large part of the protein is broken down to amino acids, amides, and ammonia. So far as is known, no data on changes in the nitrogenous constituents during flue-curing have been published, although data for cured leaf are available.

It is hoped to obtain information on any changes taking place from estimation of the classes of nitrogenous compounds in the material from the curing experiments discussed in this paper, and these will be reported in a later paper.

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## CHANGES IN VITAMIN C CONTENT AND ACIDITY OF APPLES DURING COOL STORAGE

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### Summary

Seven varieties of apples were held in cool store for from three to seven months. The vitamin C contents of the flesh, skin, and whole fruit were determined at monthly intervals. During the first two or three months all varieties, except Sturmer, rapidly decreased in vitamin C content, the skin losing it at a greater rate than the flesh. Later the rate of loss was much lower. On the other hand, the vitamin C content of Sturmer remained practically constant for seven months.

Titratable acidity of the juice decreased markedly with length of storage time. The rate of loss varied considerably with the different varieties.

There appears to be no direct correlation between vitamin C content of the flesh and the titratable acidity of the expressed juices of the apple fruits examined.

### INTRODUCTION

PREVIOUS unpublished work (E.B.K.) at the Cawthron Institute indicated that the vitamin C content of certain varieties of apples was greatly dependent on whether the fruit was examined immediately after picking or after holding at temperatures such as might be found in orchard stores. Varieties such as Jonathan, Delicious, and others decreased rapidly in vitamin C content, both in the skin and in the flesh. On the other hand, the Sturmer variety lost comparatively little of its vitamin C under the same storage conditions. Together with variations in the amount of vitamin C, large changes in the titratable acidity of juice pressed from the apples were found. It appeared to be of interest to determine what changes might occur in the vitamin C content and in the acidity of the juice on holding different varieties of apples under commercial cool-storage conditions. The present paper reports results obtained from storage of seven varieties.

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### EXPERIMENTAL

For vitamin C determinations the method described by Kidson(1) was used, except that sixteen apples per sample were taken. Separations of skin and flesh were made on the initial samples and on selected samples at the later sampling dates. The remainder of the sample after taking out the sectors for the vitamin determination was crushed and the juice used to obtain the titratable acidity figure. This is expressed in terms of millilitres of  $\frac{N}{10}$  NaOH solution required to neutralize 100 ml. of juice to the phenolphthalein end-point.

Seven varieties of apple grown in different parts of the Nelson district, mostly on the Moutere Hills soil type, were obtained. The fruit was picked about midday or early afternoon, and was packed into bushel cases direct from the trees. Alternate rows of fruit were wrapped. In the majority of orchards two cases of fruit per variety were obtained. Within a few hours of picking the fruit was placed in a commercial cool store. The conditions of storage were as follows: Delicious and Granny Smith at 31° to 33° F.; Cox's Orange, Jonathan, Sturmer, and Statesman at 36° to 38° F. Some of the varieties were held longer than they would be under commercial conditions, but all fruits analysed were sound.

At the time of picking, a sample of fruit for analysis was obtained, the determinations being made, with few exceptions, on the following day. At monthly intervals samples were taken from the cool store. On each occasion the average weight of the fruit was recorded as a check on the sampling procedure; in all cases the periodical samples agreed satisfactorily in weight with the original one for a given variety.

The orchards from which material was obtained have been denoted in the tables of data by numbers, and where more than one lot of fruit was obtained from a given orchard the samples are marked by letters, thus, "2A."

Notes on the fruit are as follows:—

*Cox's Orange*.—For orchard 1 the B sample was slightly more mature than the A sample, although the latter was well advanced in maturity. Each came from six trees in different parts of the orchard. Picked on 7/3/45.

In orchard 2 the A sample was somewhat more mature than the B sample. Picked on 7/3/45.

*Jonathan*.—In orchard 3 the fruit was obtained from eight well-grown trees. Individual fruits were even-sized and well-coloured. Picked on 19/3/45.

Fairly mature fruit with a distinctly yellow ground colour was obtained from orchard 5. Picked on 14/4/45, but not put into cool store until 16/4/45.

*Delicious*.—Fruit from the outside of the tree, well-coloured but somewhat russeted, was obtained from orchard 3 on 19/3/45. Sampled from eight trees.

In orchard 2 the fruit was less russeted than that in orchard 3. Outside fruit was again obtained. Picked on 21/3/45.

*Granny Smith*.—Good, clean fruit from trees with eight- or nine-year-old grafts was obtained from orchard 7 on 9/4/45.

Rather larger fruit than from orchard 7 was picked in orchard 5 on 10/4/45. A good, clean sample was obtained.

*Sturmer*.—Relatively mature fruit, fairly well russeted but very uniform in size, was picked from orchard 4 on 16/4/45. Leafage was sparse, so that the fruit was well exposed to the sun.

Slightly larger but less mature fruit was obtained from orchard 8 on 23/4/45. The apples were fairly free from russett, &c.

*Red Statesman and Statesman*.—Both varieties were picked from orchard 9 on 26/4/45. In each case the fruit was very free from defects.



## RESULTS

The initial data for the fruits under examination are given in Table I. For Cox's Orange the highest figures for vitamin C content have been obtained with the most mature fruit both in the flesh and in the skin, and therefore in the whole fruit. The acidity figures also are higher for orchard 1 than for orchard 2. With the exception of Granny Smith, origin of the fruit does not seem to have markedly affected the vitamin content. Statesman is lowest and Sturmer the highest for vitamin C content on the whole fruit basis. There appear to be distinct differences between the varieties in respect of the ratios of the amounts of vitamin C in the flesh and the skin. Thus Cox's Orange, Granny Smith, and Red Statesman samples, with approximately the same vitamin C content of the flesh, have given 42.5, 74.0, and 112.8 mg. per 100 g. respectively for the skin. While it is difficult to pare the fruits uniformly, the above data suggest that there are very real differences in the vitamin C content of the skin of the several varieties.

TABLE I.—VITAMIN C CONTENT, ACIDITY OF JUICE, AND AVERAGE WEIGHT OF APPLES AT TIME OF PICKING

Variety.		Orchard.	Milligrams of Vitamin C per 100 g. Fresh Material.			Titratable Acidity ml. N NaOH per 100 ml. of Juice.	Average Weight of Fruit.
			Whole Fruit.	Flesh.	Skin.		
Cox's Orange	..	1A	16.4	11.0	56.5	137.0	G.
"	..	1B	15.7	11.7	56.3	137.5	107
"	..	2A	14.5	10.5	51.1	121.5	109
"	..	2B	12.9	9.2	42.5	130.5	113
Jonathan	..	3	11.5	10.8	34.0	88.5	107
"	..	5	11.4	8.4	45.6	80.5	104
Delicious	..	2	11.8	7.4	34.5	35.0	106
"	..	3	10.8	..	..	40.5	119
Granny Smith	..	7	19.2	12.1	93.3	119.0	115
"	..	5	14.7	10.4	74.0	114.0	106
Sturmer	..	8	29.3	25.1	86.4	155.5	108
"	..	4	29.3	22.0	103.0	150.0	104
Statesman	..	9	10.1	6.5	65.1	61.5	118
Red Statesman	..	9	18.2	9.0	112.8	73.0	101

The data in Table I indicate that there are wide differences in the titratable acidity of the juices of the varieties. Sturmer has shown the highest acidity and Delicious the lowest, but there does not appear to be any direct correlation between acidity and vitamin C content of the flesh of the freshly gathered fruit.

TABLE II.—AVERAGE VITAMIN C AND ACID CONTENT OF APPLE VARIETIES AFTER COOL STORAGE

Vitamin C expressed as milligrams per 100 g. of fresh material and acidity as millilitres of  $\frac{N}{10}$  NaOH required per 100 ml. of juice.

Variety.	Part of Fruit.	At Picking.		After Three Months Cool Storage.		After Six Months Cool Storage.	
		Vitamin C.	Acidity.	Vitamin C.	Acidity.	Vitamin C.	Acidity.
Cox's Orange	Whole	14.9	131.6	7.5	76.2	..	..
	Flesh	10.6		5.2		..	
	Skin..	51.6		42.7		..	
Jonathan ..	Whole	11.4	80.7	6.4	64.8	6.4	46.5
	Flesh	9.6		4.7*		..	
	Skin..	45.6*		32.2*		..	
Delicious ..	Whole	11.3	36.5	5.3	36.2	4.5	30.2
	Flesh	7.4*		..		3.1*	
	Skin..	34.5*		..		23.0*	
Granny Smith	Whole	17.0	116.5	8.8	106.5	8.8	79.5
	Flesh	11.2		5.3*		5.8*	
	Skin..	83.6		..		..	
Red Statesman	Whole	18.2*	73.0*	10.7*	63.5*	9.5*	42.0*
	Flesh	9.0*		5.2*		5.0*	
	Skin..	112.8*		84.3*		66.9*	
Statesman ..	Whole	10.1*	61.5*	6.4*	56.0*	4.6*	36.0*
	Flesh	6.5*		3.4*		2.9*	
	Skin..	65.1*		43.8*		34.0*	
Sturmer ..	Whole	29.3	152.7	28.3	131.0	27.5	103.3
	Flesh	23.5		25.8*		23.5*	
	Skin..	94.7		89.9*		86.6*	

\* One sample only.

From the complete data selections have been made corresponding to three-monthly and six-monthly storage periods. Table II contains these data. The greater part of the loss of vitamin C in the whole fruit, the flesh, and the skin, in all varieties except Sturmer, occurs in the first three months of storage. It is also apparent from the data that there is a distinct tendency for the skin to lose its vitamin content more rapidly than does the flesh, a conclusion also reached by Fish(2) in Virginia. The complete data show that the lengths of time required for the initial value for the different varieties to fall by half were as follows: Cox's Orange, two months; Jonathan, three months; Delicious, three months (but almost as low after one month); Granny Smith, three months; Statesman, five months (but almost as low after two months); Red Statesman, four months. On the other hand, Sturmer from orchard 8 showed no loss even after seven months, and the average loss for orchards 4 and 8 was only 6 per cent. after six months in store.

## DISCUSSION

Because of the difficulty of satisfactorily presenting the complete data in tabular form, certain of the more outstanding indications have been represented graphically. Analysis of the data shows that, among the varieties tested, three types of change with time of storage are to be found. These can be arranged as follows (Table III).

TABLE III

Type.	Vitamin C Content.	Acidity.	Variety.
1	Constant ..	Decreases markedly ..	Sturmer.
2	Decreases ..	Decreases slightly ..	Delicious.
3	" ..	Decreases markedly ..	Cox's Orange.
3	" ..	" ..	Granny Smith.
3	" ..	" ..	Jonathan.
3	" ..	" ..	Red Statesman.
3	" ..	" ..	Statesman.

Conditions for the Sturmer variety (orchard 8) are shown in Fig. 1. Within the probable experimental error the vitamin C contents of the whole fruit, the skin, and the flesh are constant over the period of storage. On the other hand the acidity steadily decreases. Although the variation in

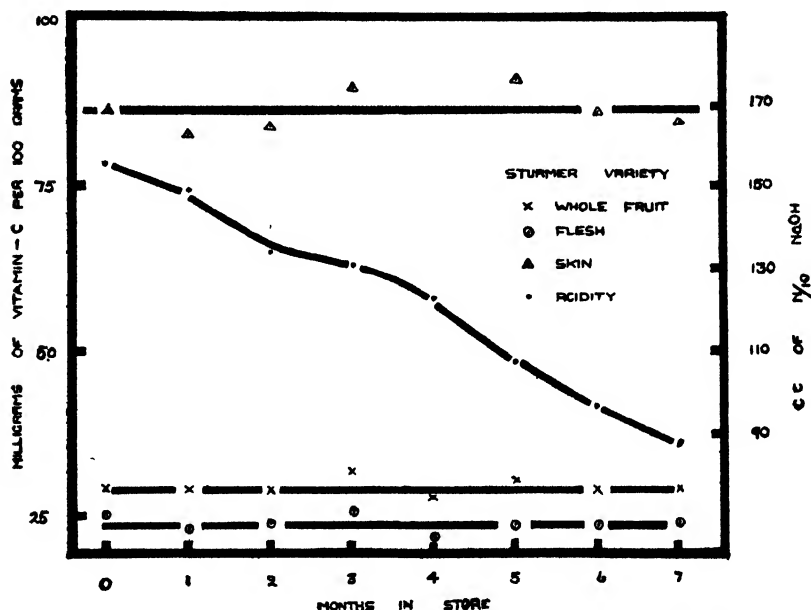


FIG. 1.—Variation of vitamin C content of whole fruits, skin, and flesh, and of acidity of Sturmer variety on cool storage.

acidity is shown by a curved line, actually a straight line can be drawn through the points, with no greater deviation than 4 per cent. from the plotted values. In the Sturmer variety there appears to be no correlation between change in acidity and vitamin C content whether in the whole fruit or in the flesh, when expressed on the basis of 100 g. of material.

In Fig. 2 are shown the variations in acidity and vitamin C content of whole Delicious fruits (type 2 of Table III). The vitamin content falls rapidly at first, but the acidity is almost constant. After three months of storage both the vitamin C and acid contents fall slowly.

An example of Type 3 is afforded by the Statesman variety, data for which are plotted in Fig. 3. The rapid loss of vitamin C from the skin and the lower rate of loss from the flesh is clearly indicated. Due to the predominating effect of the weight of the flesh compared with that of the skin, the curve for the whole fruit closely follows that for the flesh. Change in acidity is slow at first, but later becomes more marked. The shape of this curve is very similar to that shown in Fig. 2 for Jonathan variety, which also is graded as Type 3.

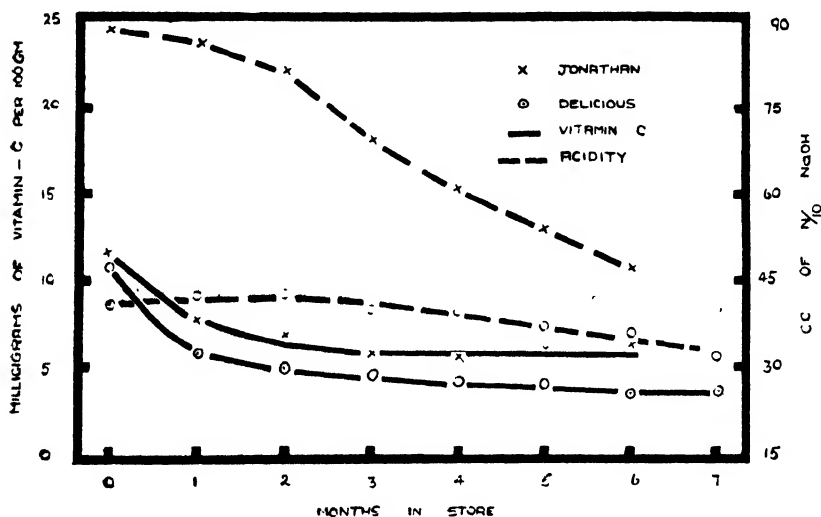


FIG. 2.—Variation of vitamin C content and acidity of Jonathan and Delicious varieties on cool storage.

While the possibility of exchange of vitamin C between the skin and the flesh should not be excluded, it seemed desirable to compare the progressive change in acidity with that of the vitamin C content of the flesh of the fruits. Data for four varieties of Type 3 are plotted in Fig. 4. The curves for variation in vitamin content of the flesh are very similar in shape, showing a rapid loss early in the storage period, but a much reduced rate of loss later on. Jonathan, Statesman, and Granny Smith give similar curves for loss of acidity, these being fairly flat at first and then falling more rapidly. With Cox's Orange, however, a straight-line relation between acidity and storage time is shown, the loss of acidity being rapid throughout. These curves provide evidence that decrease in acidity is not directly correlated with loss of vitamin C in the flesh of these apple varieties. The data for Sturmer variety in Fig. 1 should also be studied in this connection.

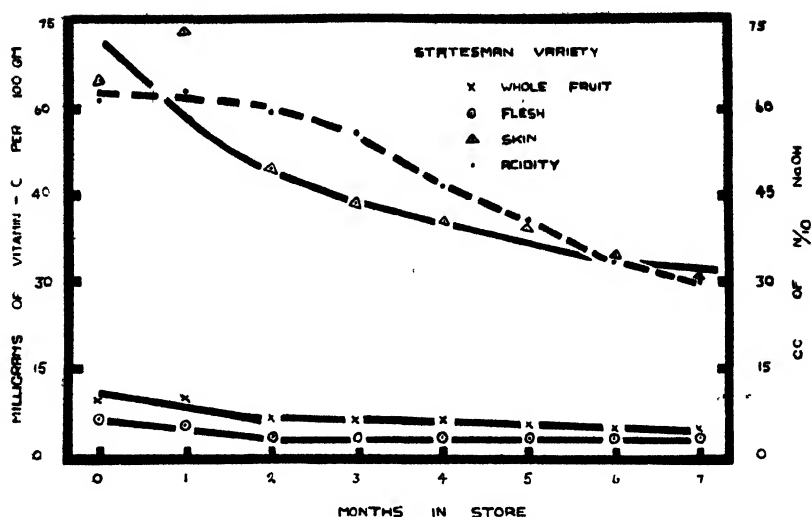


Fig. 3.—Variation of vitamin C of whole fruit, skin, and flesh and of acidity of Statesman variety on cool storage.

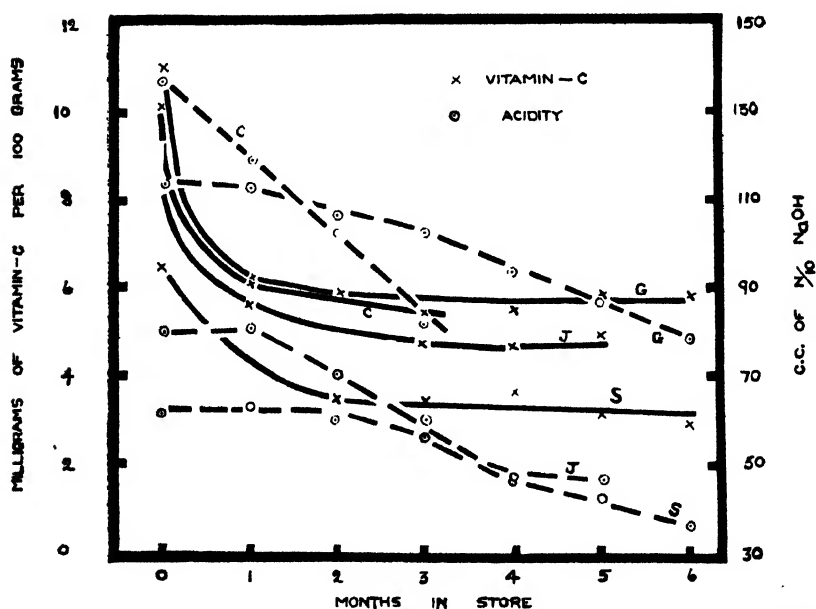


Fig. 4.—Variation of vitamin C content of flesh and of acidity of four apple varieties on cool storage.

C = Cox's Orange; G = Granny Smith; J = Jonathan; S = Statesman.

In this trial no account has been taken of the possible presence of dehydroascorbic acid in the fruit. From the work of West and Zilva(3) on the apparent synthesis of vitamin C in apples kept under cool-storage conditions it may be deduced that even with mature fruit held for as long as six months transformation of dehydroascorbic acid to ascorbic acid and synthesis of ascorbic acid (vitamin C) will occur. Thus in their experiments with Bramley's Seedling variety the vitamin C content per 100 g. of fresh fruit apparently increased (not significantly) from 18.8 mg. to 20.8 mg., while the proportion of ascorbic acid present rose from 90.2 per cent. to 95.8 per cent. of the sum of the ascorbic and dehydroascorbic acid contents. In the Nelson trial described above the rate of destruction of ascorbic acid must therefore have been greater than the rate of synthesis of ascorbic acid and of transformation of dehydroascorbic acid to ascorbic acid. On the other hand, oxidation of ascorbic acid to the dehydrocompound may have occurred. Moreover, in view of the presence of many other labile compounds such as sugars in the apple fruits, it is not impossible that the changes in vitamin C content may be related more to them than to the acids.

#### ACKNOWLEDGMENTS

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## INVESTIGATIONS ON GROWING PYRETHRUM IN NEW ZEALAND

### I. METHODS OF PROPAGATION, CULTIVATION, HARVESTING, AND DRYING

By E. E. CHAMBERLAIN, Assistant Director, and C. H. PROCTER, Technical Assistant, Plant Diseases Division, Department of Scientific and Industrial Research

*(Received for publication, 24th January, 1947)*

#### Summary

Pyrethrum has been grown successfully under experimental conditions in New Zealand for twelve years.

Plants raised from seed were extremely variable in growth type, while those propagated by rooted or soft-shoot cuttings produced even lines of plants. Satisfactory crops were secured without the use of fertilizer.

A spacing of 1 ft. between plants and 2 ft. between rows gave better results than wider spacing.

Flowers were harvested by hand, with a box stripper, and by means of a fixed comb, the last method being most satisfactory.

From small experimental crops flowers were dried successfully in the shade on scrim-covered wire-netting frames or in an electrically heated, forced-draught drier.

Percentage germination of seed saved in New Zealand was low, ranging from 22 to 42 per cent.

#### INTRODUCTION

THE possibility of growing pyrethrum (*Chrysanthemum cinerariaefolium* (Trev.) Bocc.) in New Zealand has for many years aroused considerable public interest. So many inquiries were received concerning its cultivation that in 1933 it was decided to experiment with pyrethrum cultivation under local conditions. Difficulty was experienced in securing fresh seed, which delayed work until 1934, when seed was obtained from the late Dr. A. McTaggart, Senior Research Officer, Division of Plant Industry, Council for Scientific and Industrial Research, Australia. This provided material for the investigations covered in this series of papers.

The present article deals with propagation and cultivation of pyrethrum and harvesting and drying of flowers. Subsequent papers will cover selection of strains for yield of flowers, resistance to root-rot, and habit of growth, and the effect of strains, locality, age of plants, maturity of flowers, age of flowers, &c., on pyrethrin content.



Pyrethrum is the name applied to certain species of the genus *Chrysanthemum*, some of which produce, mainly in the flowers, esters known as pyrethrins, which, while highly toxic to insects, are comparatively harmless to warm-blooded animals. The three most important pyrethrin-producing species (Mardzhanyan, 1941) are *C. cinerariaefolium* (Dalmatian pyrethrum), *C. roseum* (Persian pyrethrum), and *C. carneum* (a species which grows wild in Russia (Petrov and Ikonen, 1931)). *C. cinerariaefolium* is of the greatest economic importance and is the species used in the present investigations and referred to throughout this and subsequent articles as "pyrethrum."

Little information is available concerning the early history of pyrethrum. Most writers agree that the flowers were first used as an insecticide in Persia. The species originally employed were *C. roseum* and *C. carneum*, but about the year 1840 *C. cinerariaefolium* was produced in Yugoslavia and has since displaced the others. Yugoslavia (then known as Dalmatia) became the main pyrethrum-producing country, and held this position until 1914. Towards the end of the last century commercial cultivation was undertaken in Japan, and when the war of 1914-18 prevented production in Yugoslavia, Japan became the chief exporting country. The plant was introduced into Kenya in 1928 (Beckley *et al.* 1938) and commercial production commenced a few years later. Production increased rapidly, until now the colony is the world's largest producer. Although cultivated in many other countries, it is only in those mentioned that pyrethrum has become a crop of major importance.

#### STRAINS OF PYRETHRUM USED IN NEW ZEALAND EXPERIMENTS

During the winter of 1934 five strains of seed were secured from Australia. One of these had originally come from Switzerland while the other four were from different Japanese sources.

#### *Viability of Seed*

Ball (1944) stated that, in Kenya, seed had a germination of about 50 per cent. and lost viability rapidly. The percentage germination of pyrethrum seed harvested in Australia and New Zealand is also low. Thus germination tests of the Australian seed referred to above gave the following germination after eighteen days: strain (1), 17 per cent.; (2), 16 per cent.; (3), 31 per cent.; (4), 44 per cent.; and (5), 14 per cent. Seed harvested at Palmerston North, New Zealand, in the autumn of 1935 from three different strains gave germination counts of 30, 40, and 42 per cent. respectively. Further tests of seed harvested from two pure clone lines in 1945 gave germination counts of 22 and 25 per cent.

#### PROPAGATION

In the past it has been usual to propagate pyrethrum from seed. Martin and Tattersfield (1934), however, in order to secure uniform plants for experimental work, used clonal lines produced from root cuttings. They showed that the pyrethrin content of flowers varied with different plants, but for individual plants remained constant from season to season. Cormack (1935) propagated from selected plants, by means of root cuttings, soft-shoot cuttings and crown cuttings. He showed that it was possible to secure clones with such desirable characteristics as upright compact habit, and even and early flowering (thus facilitating harvesting), and

considered that propagation by cuttings was a practical method of increasing stocks. Drain and Shuey (1934) demonstrated that the method was also useful in selecting strains with flowers of a high pyrethrin content. Recently vegetative propagation has been used commercially in Kenya, where high-pyrethrin-producing strains have been utilized (Beckley, 1938). To ensure cross-pollination, which is essential for good yields and high pyrethrin content, it was necessary to select a large number of clones.

Under experimental conditions pyrethrum has been successfully established in New Zealand from (1) seedlings, (2) rooted cuttings, and (3) soft-shoot cuttings.



[Photo by H. Drake

FIG. 1.—Pyrethrum plants grown from seed. Compare short flower-stalks and erect compact habit of plant in foreground with long flower-stalks and spreading habit of surrounding plants. Photo taken twelve months after setting plants out in the field.

#### (1) *Seedlings*

Seed was sown in seed-boxes in a heated glasshouse during August, and young plants pricked out into a seed-bed at the end of September. Seedlings were spaced approximately  $1\frac{1}{2}$  in. apart and were shaded for the first ten to fourteen days. In November the plants were set out in the field. Such plants grew well and sometimes produced a few flowers in the first autumn. They came into full flower in the following November and December. Observations made during the flowering period showed that plants raised from seed varied greatly in size, habit, amount and colour of foliage, shape of leaves, length of flower-stalks, number and size of flower-heads, total yield of flowers, evenness of flowering, and susceptibility to root-rot (Fig. 1).

### (2) Rooted Cuttings

Pyrethrum plants can be split into numerous portions each bearing roots (Fig. 2 (a)). Their number depended on strain, age, and condition of the parent plant, varying from less than ten to over one hundred. Division of plants required such care that one person could prepare only 100 cuttings in an hour. Best results were obtained when cuttings were taken before mid-September, though a percentage strike was obtained from mid-June to early October. They were either set out directly into permanent positions in the field or pricked out into beds. The latter method proved more satisfactory, as cuttings could be given more attention, with the result that percentage survival was much higher, and better and more uniform plants obtained.



[Photo by L. H. Wright.]

Fig. 2.—Pyrethrum cuttings: (a) rooted cuttings prepared for planting; (b) soft-shoot cuttings ready for striking in sand.

Unless conditions of soil and season were favourable, planting directly to the field led to uneven growth. Cuttings raised in beds, however, and set out in the field during November and December produced even, vigorous plants. With both methods a few flowers were sometimes produced in the autumn, but plants did not come into full bearing until November–December of the year following setting out.

A modification of this method was to divide plants into sections (Fig. 3) and set them out into their permanent positions. They were found to establish readily.

### (3) *Soft-shoot Cuttings*

From old plants and plants of certain strains only a few cuttings with roots could be secured. Such plants, however, yielded numerous soft shoots suitable for cuttings (Fig. 2 (b)). These were removed, trimmed, and struck in sand at the rate of 350 cuttings per seedling-box of 18 in.  $\times$  12 in.  $\times$  3 in. deep (Fig. 4). A well-grown plant yielded up to 350 cuttings, and one person could prepare and plant about 200 per hour. Cuttings were taken during June, July, and August. Under favourable conditions a strike of over 90 per cent. was obtained. When well rooted, cuttings were pricked out



[Photo by L. H. Wright.]

FIG. 3.—Portion of a well-grown pyrethrum plant which has been divided up into a number of sections. Such divisions may be set out in permanent positions to establish a new area.

into beds, the spacing being 1½ in. either way. They required some protection from direct sunlight during the early stages. Plants were transferred from beds to the field during November and December and came into full bearing twelve months later. Using this method, very even stands were obtained in the field (Fig. 5).

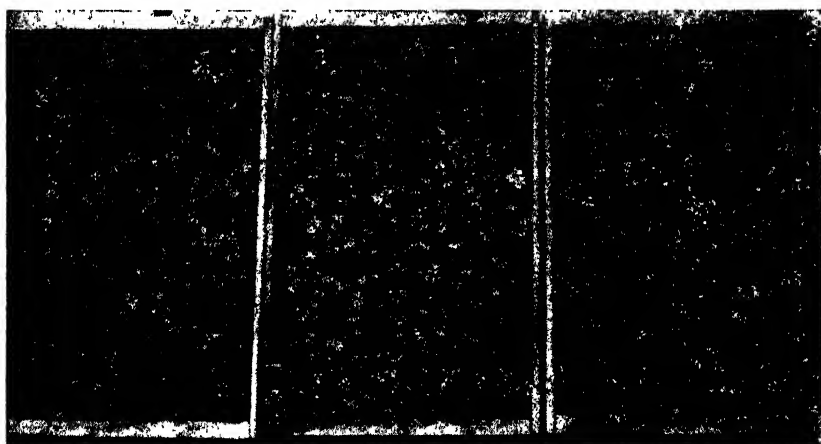
Where methods of vegetative propagation were used the progeny of single plants were kept separate so that clonal lines were obtained. No variation was observed within clones. Variation between clones, however, was as great as that between individual plants in lines raised from seed. Differences in leaf shape and in plant habit are shown in Figs. 2 and 3.

## MANURING

Application of fertilizers to pyrethrum crops has received a considerable amount of attention. It has been claimed by some that manuring is detrimental to the crop, but in poor soils such as that existing on the island of Hokkaido in Japan the use of manures is apparently necessary (Gnadinger, 1933). Recent work has shown that, although the manurial requirements of the crop are small, fertilizers may be applied with advantage (Martin *et al.* 1939; Ball, 1944). In our experiments no fertilizers were applied either at planting or subsequently.

## SPACING OF PLANTS IN THE FIELD

In commercial crops there is considerable variation in spacing of plants. Thus in Yugoslavia they are grown roughly 10 in. to 12 in. apart, and in Japan seedlings are set out at 7 in. to 12 in. with 12 in. to 24 in. between rows (Gnadinger, 1933). Spacing in Kenya varies from 15 in.  $\times$  15 in. to 24 in.  $\times$  30 in., according to locality and soil type (Beckley *et al.*, 1938). Under experimental conditions in Australia plants were set out in rows 18 in. apart with 12 in. between plants (McTaggart, 1934), and in Rhodesia at 6 in. to 12 in. with 18 in. to 24 in. between rows (Arnold, 1939).



[Photo by H. Drake.

FIG. 4.—Soft-shoot cuttings of pyrethrum. Each box contains 350 cuttings taken from a single one-year-old plant and struck in sand. Note variation of foliage type between cuttings in the different boxes.

In the United States of America Evans *et al.* (1935) experimented with effects of spacing interval in the row upon yield of flowers. Plants were spaced at 9, 12, 15, 18, 21, and 24 in. apart in rows 30 in. apart. They found that closer spacings gave a significantly greater yield per acre than did wider spacings. They found also that close spacing induced upright stems, with a decreased tendency to spread and lodge.

In New Zealand most plantings have been carried out with a spacing of 12 in. between plants and 24 in. between rows. In one planting spacing was increased to 18 in. between plants and 30 in. between rows. No comparative trials were made, but observations and gross weights of crop suggested that the wider spacing did not yield a greater weight of flowers per plant, and consequently yield per acre was much reduced. At the wider spacing there was a greater tendency for plants to spread and for flower-heads to lodge.

### CULTIVATION AND WEEDING

Shallow cultivation was practised, the crop responding well to such treatment. It was found necessary to keep the crop as free as possible from weeds. When plants were small, cultivation between rows with a Planet Junior was possible, but in a well-grown crop it was necessary to use a hoe.

### CUTTING BACK

Overseas, the extent to which plants should be cut back after harvesting has received some attention. In Kenya it was found that cutting of foliage caused a serious reduction of crop in the following season, and that highest yields were obtained when only old flower-stalks were removed (Ball, 1944).

In New Zealand experiments flower-stalks were cut with a sickle during harvesting, and as far as possible no foliage was removed. Out-of-season flowers which appeared in the autumn were also removed with a sickle.



Photo by H. Drake

FIG. 5.—Clonal lines of pyrethrum. Plants raised from soft-shoot cuttings. Note difference in growth between the three strains: (1) top left, (2) three rows left centre, and (3) two rows right. Photo taken nine months after setting plants out in the field.

### HARVESTING OF FLOWERS

Under commercial conditions pyrethrum flower-heads have been either hand-picked or flowers cut with a sickle and heads removed with a comb. In Kenya, where the flowering period extends over ten months of the year (Beckley, 1938), hand-picking is a necessity. In Japan larger growers used the comb method (Gnadinger, 1933). Both methods are costly in labour, and in recent years workers in the United States of America have sought to lower harvesting costs by using semi-mechanical methods or mechanical strippers (Sievers *et al.*, 1941). From the authors' descriptions it would appear that a satisfactory type of mechanical harvester has been evolved.

During the first harvest of the experimental crop in New Zealand all flowers were hand-picked. In the second season a box-stripper consisting of a comb with canvas receptacle was used. This required two men to operate, one holding the stripper and the other adjusting the flower-heads on to the comb. The box-stripper method, although an improvement on hand-picking, proved to be slow and tedious. In the following year a fixed comb was set up and flowers cut with a sickle were stripped on the comb and heads collected on a canvas sheet. This last method proved quite satisfactory for handling a small experimental crop.

#### DRYING OF FLOWERS

It has been the practice in Yugoslavia and Japan to air-dry flowers. In the former country drying was carried out in the shade if possible, but, when rain threatened, flowers were dried as quickly as possible in the sun. In Japan drying in the sun was the general practice (Gnadinger, 1933). Such methods are practicable only in countries where there is a dry harvesting season. In Kenya, where flowers are picked during the wet season, kiln-drying has been developed (Beckley, 1937; Walker, 1940).

Even in Yugoslavia and Japan air-drying was not very satisfactory, as variations in weather often resulted in development of mould which caused discoloration of flowers and lowered their pyrethrin content. Kiln-drying is much superior as it produces a uniform product, the flowers being bright in colour and having a consistent pyrethrin content.

Under New Zealand conditions drying of flowers in the open is not practicable since the weather is usually unsettled during the harvesting period of late November and early December. At first, flower-heads were laid out on benches or spread on the floor of a barn to dry. This method was unsatisfactory, for, during periods of dull or wet weather, it was almost impossible to prevent development of mould, in spite of spreading flowers into very thin layers and turning frequently. The method was soon abandoned, and flowers dried on wire-netting frames covered with scrim. It was necessary to keep the layers thin and to turn frequently in dull or wet weather. By exercising much care this method was found satisfactory for drying flowers from small experimental areas.

Smaller samples have also been successfully dried in an electrically heated drier with forced draught.

#### NEED FOR SELECTION

Seedlings from five strains of seed were set out in the field in November of 1934 and made good growth during the summer. They produced a small crop of flowers during the autumn, and the extreme variation in plants at this stage emphasized the need for selection.

From the outset it was evident that, with the high labour-costs ruling in New Zealand, pyrethrum could not be economically produced in the Dominion under methods similar to those employed in other countries. To make production possible it would be necessary both to increase the value of the crop per acre and to use a mechanical means of harvesting. Value of crop per acre could be raised by increasing the yield of flower-heads and by improving their pyrethrin content. To permit of mechanical harvesting, plants would require to (1) be even in size, (2) produce flower-stalks of the same length, (3) be upright in habit of growth, and (4) come into flower at the same time. Resistance of plants to root-rot would also have to be considered.

Martin and Tattersfield (1934) had already shown that an even line of plants could be obtained by striking a number of root cuttings from a single plant. This suggested the possibility of selecting plants for type and increasing them by vegetative propagation. Details of work on the selection of pure clone lines will be given in the next of this series of articles.

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(To be continued)

## TEMPERATURE AND HUMIDITY CONTROL IN CHEESE-CURING ROOMS

By T. R. VERNON, Dairy Research Institute (N.Z.), Department of  
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[Received for publication, 21st October, 1946]

### Summary

(1) Similar cheeses from the one vat of milk were stored for the usual fourteen-day period in three different types of curing-room:—

- (a) An uninsulated room.
- (b) An insulated room.
- (c) An insulated room controlled automatically at a temperature of 55° F. and a humidity of 85 per cent.

The trials were carried out over two dairying seasons.

(2) The cheeses from the controlled room were of better quality both at fourteen days and at three months than the corresponding cheeses from the other rooms, and they regularly suffered less shrinkage (to the extent of 0.25 to 0.5 per cent.) during the time they were under controlled conditions. Later after three months storage at 45° F. with no control of humidity they finally showed the same total shrinkage as the other cheeses.

(3) The suppression of mould-growth in the controlled room was not complete, although it was adequate for practical purposes. A lowering of the relative humidity to 80-82 per cent. might be advisable.

(4) The extra cost of plant for the controlled room is partly offset by the simpler construction of the room itself, and the cost of operation is probably offset by the saving in shrinkage.



NEW ZEALAND Cheddar cheese is held in a curing-room at the factory for at least fourteen days before it is packed for export. During this storage period the fresh cheese curd is undergoing very rapid changes under the influence of the proteolytic enzymes, hence the importance of controlled temperature and humidity conditions in the curing-room is greater than at any subsequent stage in the "life" of the cheese.

Maintenance of the temperature within a certain range is necessary to provide optimum conditions for the enzymatic changes in the cheese curd. Too high a temperature leads to abnormal protein breakdown and to excessive mechanical settling with loss of shape of the cheese. Too low a temperature delays ripening. Fluctuations in temperature by causing excessive contraction and expansion lead to mechanical fractures in the body of the cheese.

Too dry an atmosphere in the curing-room will give excessive shrinkage of the cheese due to evaporation of moisture, and consequently a lower monetary return to the company. On the other hand, too high a humidity (especially when accompanied by a high temperature) will encourage mould-growth on the rind of the cheese. The mould may then penetrate into the interior of the cheese through cracks or plug holes and cause spoilage. But even if the mould merely forms a coating on the rind it is objectionable to workers who have to handle the cheese, and inhalation of the spore-dust over a long period may be injurious to health.

From these several points of view it is therefore desirable that curing-rooms at cheese-factories should be designed so as to provide optimum conditions for cheese-ripening. Many rooms in New Zealand factories are far from satisfactory at present, and the work described in this paper was undertaken with the object of finding out how best to improve the general design of cheese-curing rooms. Originally the main object of the work was to specify conditions which would minimize mould-growth on cheese, but it soon became evident that that was only one of the advantages to be gained by controlling temperature and humidity. Saving of shrinkage in particular seemed to be a feature which would offer an inducement to cheese-producing companies to improve their curing-rooms.

#### DEFINITION OF OPTIMUM CONDITIONS

It will be clear from the foregoing that optimum atmospheric conditions in the curing-room are a matter for compromise. High humidity reduces shrinkage but encourages mould-growth. Low humidity reduces mould-growth but causes excessive shrinkage. It is necessary, therefore, to strike a balance between these opposing effects and at the same time maintain a constant, suitable temperature. Preliminary experiments indicated that mould did not readily develop on the rind of freshly made Cheddar cheese when the relative humidity of the surrounding atmosphere was kept below 80 per cent. and that between 80 and 85 per cent. R.H. mould-growth was kept to a satisfactory minimum. This, then, sets the upper permissible limit for humidity. Cheese-ripening proceeds normally at a temperature between 55° and 60° F. Since mould-growth is restrained by low temperature the curing-room should thus be maintained at an upper temperature limit of 55° F.

Optimum values to be aimed at in the experiments were therefore provisionally set at 55° F. and 80-85 per cent. R.H.

## EXISTING CURING-ROOMS

The design of cheese-curing rooms has not hitherto received much attention in New Zealand. In most of the rooms atmospheric conditions are virtually uncontrolled, and both temperature and humidity undergo wide variations. During hot weather the high temperature in the curing-room tends to govern the methods of cheese-manufacture employed by the manager in that the cheese has to be made to "stand up" to conditions in the curing-room. The only methods available for partial control of temperature and humidity are ventilation through the windows or through trap-doors in the ceiling, and heating by steam-pipes in cold weather. The only control for mould-growth consists in the burning of sulphur or the use of formalin. Existing curing-rooms fall into one of the following classes:—

- (1) *Uninsulated*.—These older curing-rooms are usually built of wood. They are fitted with single-glazed windows which provide light and ventilation. High ceilings with adjustable trap-doors also help with the ventilation. There is usually no means of temperature-control.
- (2) *Insulated*.—(a) Without refrigeration; (b) with refrigeration, manually controlled.

Until recently the use of insulation and double windows represented the only improvement in curing-room design over the uninsulated wooden room. The more modern rooms of this improved type are built of brick or concrete with 2 in. (occasionally 4 in.) of cork insulation on walls and ceiling. The older insulated rooms are of wood with 4-6 in. of pumice or sawdust for insulation.

In a few rooms a mechanical refrigerating-plant is used for cooling in hot weather, but the freezing unit is manually controlled, and consequently it is difficult to maintain a constant temperature in the room. Furthermore, a lowering of temperature without coincident humidity-control tends to stimulate mould-growth.

The insulated curing-room does help to maintain cooler curing conditions in hot weather, but it leaves much to be desired. Mould is more troublesome than in an uninsulated room, and if the room does get warm it takes longer to cool down because of the insulation. So from all points of view it was evident, when work on curing-room design was projected, that both temperature and humidity had to be controlled if completely satisfactory results were to be obtained.

## THE FIRST AIR-CONDITIONED CURING-ROOM IN NEW ZEALAND

One dairy company was faced with the necessity of building a new curing-room, and the directors were persuaded to construct it on new lines and to install apparatus for the control of temperature and humidity. This was an important step likely to affect the whole industry, so the opportunity was taken, with the co-operation of the factory, to carry out experiments to determine the effects of the controlled curing-room conditions on the cheese.

The controlled room, of reinforced concrete, was well insulated and was fitted with equipment for automatic temperature and humidity control. Ducts were used to distribute the conditioned air evenly throughout the room. The building had a low ceiling, and no windows whatever were provided.

Figure 1 is a sketch-plan of the general lay-out. The ducting was suspended from the ceiling, and adjustable ports were arranged so as to give good air distribution as indicated by arrows. An adjustable port on the exterior wall behind the fan permitted air from outside the room to be drawn in as desired. Humidity was lowered by means of an electric heater operated by the humidistat, the excess moisture being condensed on cooling coils operated by the thermostat. Steam injected into the duct system (humidistat controlled) raised the humidity when necessary.

#### TEMPERATURE AND HUMIDITY VARIATION IN PRACTICE

The controlled curing-room described above was compared with insulated and uninsulated rooms, without controls, situated at cheese-factories all in the same district.

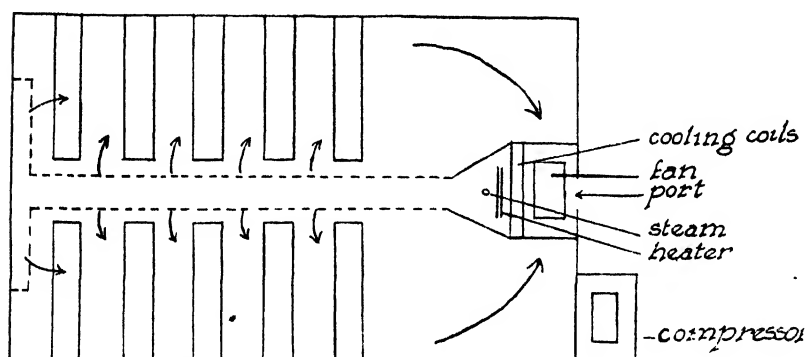


FIG. 1.—Sketch-plan of controlled (air-conditioned) cheese-curing room.

Thermographic records were kept for the three types of curing-rooms throughout the cheesemaking season. The factory managers also recorded temperatures at 9 a.m. and 4 p.m. daily. Table I shows the maximum, minimum, and mean temperatures in the curing-rooms.

TABLE I.—TEMPERATURE IN CURING-ROOMS (° F.)

Month.	Insulated.			Insulated.			Controlled.		
	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.
October .. ..	64	54	58	60	50	54	56	53	54.5
November .. .	69	58	63	66.5	55	61	57	53	55
December .. .	74	59	66	69	60	64	57	54	55.5
January .. .	74	61	67	71	59	65.5	57	54	55.5
February .. .	71	58	64	69	56	62	57	54	55.5
March .. .	71	57	64	70	55	62	57	54	55.5

The temperatures in Table I refer to three factories situated within a few miles of each other, and while they illustrate the effect of insulation and control they do not bring out the great variations in curing-room temperatures that occur throughout New Zealand. Temperatures of 80° F. and more

have been observed in curing-rooms. Table II shows the maximum daily variation in temperature during the season following that referred to in Table I.

TABLE II.—CURING-ROOM TEMPERATURES : MAXIMUM DAILY VARIATION (° F.)

Month.	Outside.	Uninsulated.	Insulated.	Controlled.
November .. .. .	35	12	6	4
December .. .. .	28	13	9	3
January .. .. .	35	15	7	3
February .. .. .	35	14	7	3
March .. .. .	37	17	9	3

Measurements of humidity were made throughout the season by means of a whirling hygrometer. Figure 2 shows the daily readings of wet- and dry-bulb thermometers in an uninsulated and a controlled room on fourteen

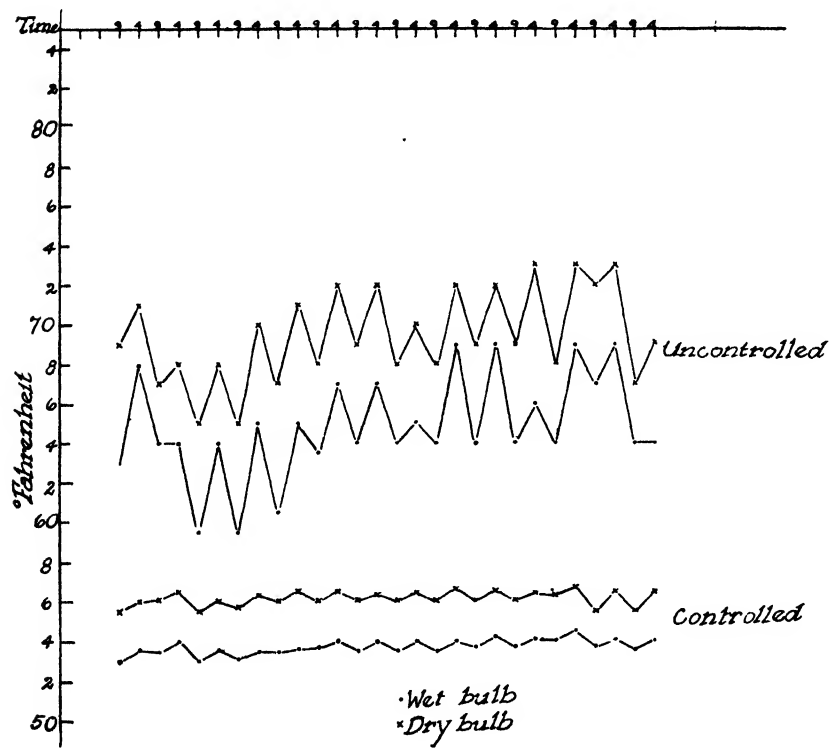


FIG. 2.—Wet- and dry-bulb temperatures in controlled (air-conditioned) and uncontrolled cheese-curing rooms for fortnight ending 26th January, 1940.

consecutive days, readings being taken at 9 a.m. and 4 p.m. daily. During the season the humidity in the uninsulated room varied from 50–95 per cent., with wide daily fluctuations, particularly in the autumn, when night

temperatures were low and day temperatures fairly high. In the insulated (but uncontrolled) room the daily variations were not so great and humidity was generally higher than in the uninsulated room. The range of variation during the season was 61-94 per cent. R.H.

#### INFLUENCE OF THE THREE TYPES OF ROOM ON THE CHEESE

With the co-operation of the factories concerned, an investigation over two dairying seasons was made into the effect upon cheese of storage for fourteen days in an uninsulated, an insulated, and an insulated and controlled curing-room. Cheeses from a vat in one of the factories were transported in their hoops to the various curing-rooms. The cheeses were weighed as taken from the hoops and again after fourteen days storage. Notes on the presence or absence of mould-growth on the cheeses were made when they were graded for quality at fourteen days. Records of temperature and humidity in the curing-rooms were kept throughout the two seasons.

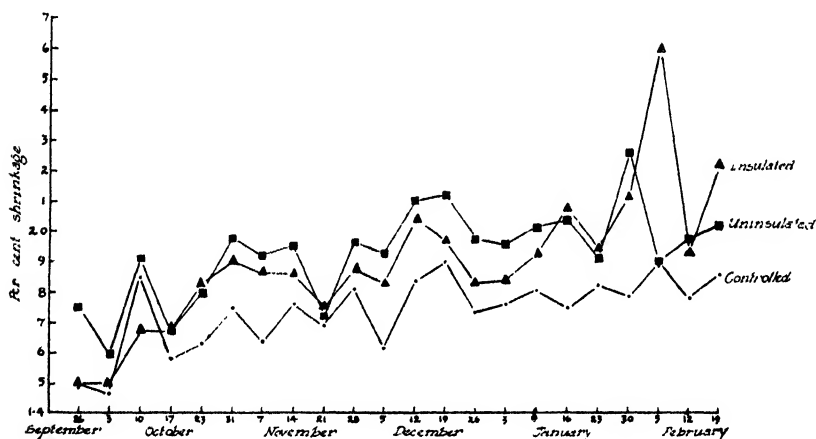


FIG. 3.—Cheese shrinkage in three types of curing-room for season 1939-40.

(1) *Shrinkage*.—Although the loss in weight of cheese during the curing period is not due to a simple drying process, obviously both temperature and humidity are important factors. Shrinkage losses are greatest under conditions of high temperature and low humidity, and it was to be expected that control of temperature and humidity would reduce shrinkage. From the results plotted in Figs. 3 and 4 several important points emerge.

The shrinkage losses in the controlled room were less than those in the other two rooms. The saving amounted to 0.25-0.5 per cent. (in a factory with a yearly output of 400 tons—this is equivalent to 1-2 tons of cheese per year). The general trend of all three curves is similar. This fact, together with the variability of the shrinkage in the controlled room, indicates some factor in the nature of the cheeses themselves which affects shrinkage even more than temperature and humidity. Further work is required on this point. While controlled curing-room conditions result in a definite reduction in shrinkage losses, this reduction is not maintained

during subsequent storage. After a further three months at 45° F. (no humidity-control) shrinkage losses were approximately the same with cheeses from all three curing-rooms—viz., controlled, 4.56 per cent.; insulated, 4.66 per cent.; uninsulated, 4.61 per cent. This result draws attention to the desirability of air-conditioning cheese-storage space in ports and on ships as well as in the dairy factory, so that the cheese may be kept under ideal conditions until it reaches the market. Under present conditions some of the benefit derived from a controlled curing-room is negated (and in particular the saving in shrinkage is lost again) by storage of the cheese under uncontrolled humidity conditions after it leaves the dairy factory. Since, however, the factory is paid on the packed weight of the cheese, the saving in shrinkage due to an air-conditioned room brings a monetary gain to the individual factory and helps to pay for the costs of operation of the air-conditioning plant.

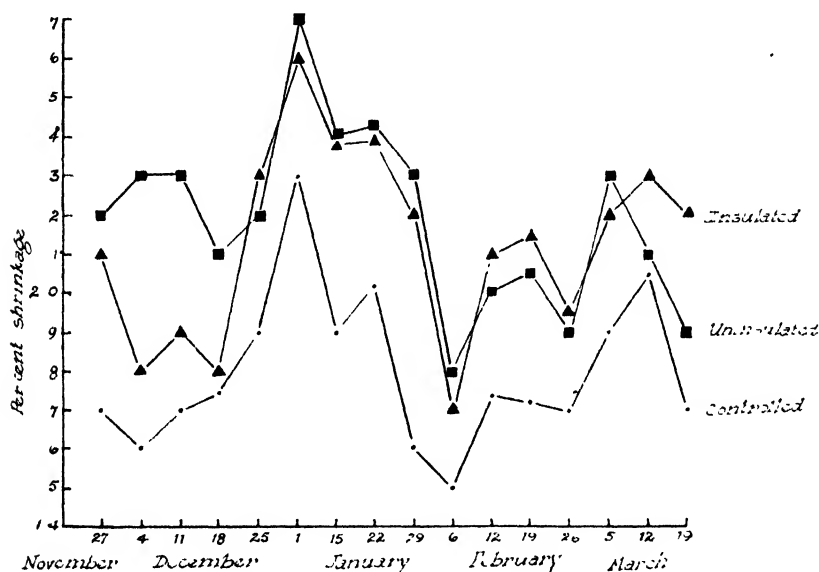


FIG. 4.—Cheese shrinkage in three types of curing-room for season 1940-41.

(2) *Quality*.—An important factor in these experiments which tended to obscure the full effects of controlled conditions on cheese-curing was the nature of the experimental cheeses. On account of transport difficulties it was necessary to obtain them from the factory which used the uninsulated curing-room. They were consequently not quite normal because they had been specially made to withstand the variations in temperature and humidity which occur in an uncontrolled room. In spite of this, however, the grading of the experimental cheeses at fourteen days and at three months showed that controlled conditions in the curing-room during the first fourteen days had a beneficial effect on quality. The tests were carried out over a period of seventeen weeks from November to March, two cheeses being graded from

each curing-room each week. The results are given in Table III. The grading was done by the official Government Grader at the port of export without knowledge of the significance of the cheese markings. On eleven occasions out of the seventeen the cheeses from the controlled room were considered to be smoother in body and closer in texture than corresponding cheeses from the uncontrolled rooms. Compared with cheese from the uninsulated room, cheese from the controlled room was awarded at the fourteen-day grading:—

- 1 point more on 2 occasions.
- $\frac{3}{4}$  point more on 3 occasions.
- $\frac{1}{2}$  point more on 7 occasions.
- $\frac{1}{4}$  point more on 1 occasion.

TABLE III.—EFFECT OF CONTROLLED CURING-ROOM CONDITIONS ON CHEESE QUALITY

Graded at Fourteen Days.			Regraded at Three Months.		
Uninsulated.	Insulated.	Controlled.	Uninsulated.	Insulated.	Controlled.
92.0	92.5	92.5	92.5	93.0	93.0
92.0	92.0	92.5	92.0	92.0	92.5
91.5	91.5	92.0	91.5	92.0	92.25
92.0	92.0	93.0	91.0	91.0	92.0
91.5	91.5	92.25	91.5	91.5	92.0
92.0	92.0	92.0	91.5	91.5	91.5
92.0	92.0	92.5	92.0	92.25	92.5
92.5	92.5	93.0	92.5	92.5	93.0
92.0	92.0	92.5	92.0	92.0	92.5
91.5	91.5	92.5	92.5	92.0	92.5
92.0	92.0	92.75	92.25	92.25	93.0
91.5	91.5	91.5	91.0	91.0	91.0
91.5	91.5	92.25	91.5	91.5	92.0
91.0	91.0	91.5	91.0	91.0	91.0
92.0	92.0	92.0	92.0	92.0	92.0
92.25	92.25	92.5	92.25	92.25	92.5
92.0	92.0	92.0	92.0	92.0	92.0
Av. 91.84	91.87	92.31	91.76	91.86	92.18

No difference was detectable on four occasions, but on two of these all the cheeses were marked down for some special defect. In round figures, at fourteen days the cheeses from the controlled room were half a point better than those from the uncontrolled rooms. At three months the difference was smaller but still significant. The grader's general comments were as follows: "There was a slight preference for the body of the cheeses from the controlled room. These were mostly 'smoother rubbing' and 'meatier,' and the texture was inclined to be closer. Cheese carrying a shade too much acid showed to better advantage from the controlled room, the body 'rubbing smoother.'"

(3) *Mould*.—Estimations of the extent of moulding of the cheese were made at fourteen days and after cold storage for three months. In one set of trials, cheeses in the controlled room were practically free from mould, but in the other series slight moulding was evident. Cheeses from all three rooms showed relatively little mould in fourteen days. The uninsulated room gave least mould (humidity generally low). The controlled room occupied a midway position, while most mould developed in the insulated room (humidity occasionally high). Mould-growth was correlated with humidity.

The development of mould in the controlled room may be partly explained by the initial difficulty experienced in adjusting the humidity-controls and partly by the fact that the new concrete of the room had not had time to dry out thoroughly.

The results indicate that controlled humidity limits mould-growth on cheese to a satisfactory degree, but that to give a greater margin of safety it might be advisable to aim at a humidity of 80-82 per cent. rather than the 85 per cent. aimed at in the trials.

(4) *Cost.*—Unfortunately, it was not possible to get an accurate measurement of the cost of operation. The power used passed through the general factory meter, and a number of factors prevented a reliable estimate from being obtained. As nearly as can be judged from the figures available, the running-costs amounted approximately to £1 per 10 tons of cheese. This must be regarded as only a very rough estimate.

In estimating costs it is necessary, in addition to calculations with regard to capital expenditure, depreciation, repairs, maintenance, and operating costs, to take into account the cost of the curing-room itself. The insulated curing-room with high ceiling and double windows is a costly structure and of doubtful value, as the tests described above have shown that the advantage of insulation *per se* are not great. On the other hand, the design of a controlled room is simpler. The plain, insulated walls are easier to construct and are more effective insulators than walls broken by a number of windows. Control of temperature and humidity eliminates the expense of double glazing and high ceilings.

Thus the controlled room is cheaper to build, the saving going far towards covering the cost of the controlling equipment. The saving in shrinkage probably covers the operating-costs. Thus any improvement in quality and any reduction of mould is clear gain. In addition, there is the added advantage that the cheesemaker does not have to make the cheese to suit high and varying curing-room temperatures.

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## LAND-CRESS TAINT IN CREAM AND BUTTER

### III. RELATION OF CONDITIONS OF FEEDING LAND-CRESS TO COWS TO INCIDENCE OF LAND-CRESS TAINT IN CREAM AND BUTTER

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*(Continued from p. 315)*

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#### Summary

Cress taint was present in cream from a cow drenched with juice from 1 lb. to 15 lb. of land-cress-plant material thirty and sixty minutes before milking. Voluntary consumption of as little as 2 oz. of cress by cows being fed in stalls caused land-cress taint in the cream from milk drawn within thirty minutes after ingestion of the cress.

Forced feeding of 1 lb. to 5 lb. of minced land-cress mixed with meals gave strong cress taint in the night's cream, and frequently cress taint in the morning's cream. Cress taint appeared in the milk and cream within thirty minutes of forced administration of cress to the cow. When cows were forcibly fed with cress at noon and were milked at 3 p.m. and 9 p.m., the strongest cress taint occurred in the cream from the 9 p.m. milking. Cress taint frequently occurred in the morning's milk and cream from cows fed cress at noon the previous day, but the intensity of the taint was much lower than for the evening's milk and cream.

The effect of cress ingestion on the flavour of milk and cream varied for different cows on any one day, and with the individual cow from day to day.

Admixture of clover with the cress fed to cows did not affect the intensity of land-cress taint in the cream.

Land-cress hay caused a cress taint in the cream, but the effect was much weaker than with the fresh plant.

Field grazing trials showed that removal of the herd from cress-infested pastures at 12 noon was not completely effective in preventing cress taint in the cream, but on some days cress taint was absent from the herd supply, and on all days the intensity of the taint was greatly reduced. It is recommended that all care be taken on dairy-farms to prevent cress infestation, by maintaining a dense pasture sward, by autumn sowing of new pastures in preference to spring sowing, and by use of a first-quality grass- and clover-seed mixture. Where cress infestation occurs, the area preferably should be grazed with dry stock. If this is not possible, controlled grazing—i.e., removal of the cows from the area not later than noon each day—should be practised.

LAND-CRESS taint in milk and cream, due to ingestion of the land-cress plant (*Coronopus didymus*) by milking-cows, has been described in a previous paper(1). Removal of the taint from cream by processing in the factory has not been generally successful, as will be shown in a subsequent paper. The best approach to a method of control appears to be careful supervision of the grazing conditions of the herds on the farms. The present paper gives the results of some investigations on the relation of these grazing conditions to appearance and intensity of land-cress taint in milk and cream.

## TRANSMISSION OF TAINTS FROM FEEDS TO MILK, AND THEIR ELIMINATION

The time interval between consumption of a tainting food by a cow and the first appearance of taint in the milk may be very short. Garlic taint, for example, has been noted in milk one minute after the cow had consumed garlic (over a period of ten minutes) and was quite strong in ten minutes. The quick transfer of taint to the blood-stream may occur either via the inhalation during mastication of the plant material or via the return juices from the rumen, which, as Schalk and Amadon(2) have shown, is constantly being flooded by liquids from the second stomach. These liquids rapidly pass to the third and fourth stomachs, where they can be absorbed. The actual time interval before the taint appears in the milk varies with the amount of tainting food given, the condition in which it is given—*e.g.*, solid or liquid—the nature of the tainting substance, and the individuality of the cow.

Most taints can be eliminated by the cow within a period of a few hours after ingestion of the tainting food. They are either destroyed by conversion to odourless substances, or are excreted in the faeces and urine, or are dispersed in the exhalation. The breath of cows that have consumed penny-cress (*Thlaspe arvense*), for example, has been reported(3) to be irritating to the eyes of a person standing nearby, and the breath of cows that have consumed onions has a strong onion odour. When cows were forcibly fed with land-cress, as described later, their breath had an objectionable odour. The cow can eliminate taints even from preformed milk in the udder, apparently by diffusion from the milk in the alveoli back to the blood-stream.

## CONTROLLED GRAZING AND INCIDENCE OF TAINTS IN MILK

Riddet *et al.*(4), Davies(5), and Marryat(6) have shown that transference of cows from clovery pastures some hours before milking greatly reduces the incidence of clover taint in the milk, and the work of Davies and Marryat demonstrated clearly that there is no loss of production either of milk or of butterfat when cows are transferred to a bare paddock several hours before the evening milking, provided that the pasture grass in the grazing area is sufficiently long to allow the cow to gather enough food during the shorter period available for feeding. Davies recommended that the cows should be transferred to the milking-yard, whereas Marryat recommended use of a bare paddock because of its greater comfort. They agree that the area should have both good water-supply and shade.

Night's milk is always more heavily tainted than morning's milk, due to the effect of the grazing habits of the cows. Levy(7) has shown that cows graze at all hours during the day, but normally not after midnight during the night interval between milkings. This is fortunate, as, owing to the practical difficulties of shifting cows from a feed-taint-producing pasture at night, controlled grazing during the night interval appears not to be feasible. It is not usual for all pastures on a farm to be infested with land-cress. Control of the taint by control grazing is thus simplified, because the cows can be removed to the clean pasture instead of to the yard or bare paddock, if this proves more convenient.

Allo and McDowall(8) have already discussed the control of land-cress taint as a farming problem, and have recommended the farmer "(a) to do his utmost to prevent the growth of cress in his dairy pasture by a carefully planned system of pasture management, and (b) if, in spite of his efforts, or through any other cause outside his control, cress invasion occurs, to initiate a system of controlled grazing on the infested areas."

These recommendations are based on grazing trials first undertaken by Walker at Tauranga and later continued by the authors at the Dairy Research Institute (N.Z.) and at Tauranga and Te Puke, and it is the purpose of the present paper to give a detailed account of the trials.

#### DRENCHING OF COWS WITH LAND-CRESS JUICE

Campbell(9) in 1937 drenched cows with juice obtained from the land-cress plant by means of a juice-extractor, and milked the cows at one-half-hour and one-hour intervals after drenching. The milk was separated and the creams were graded. The results are given in Table I.

TABLE I.—RESULTS OF DRENCHING COW "MINX" WITH LAND-CRESS JUICE  
(After Campbell)

Date.	Weight of Cress from which Juice extracted.	Time of Drenching.	Time Intervals between Drenching and Milking.	Grading Remarks on Cream.
21/11/37 ..	15 lb. mature and seedy	Night milking	1 hr. ..	Strong sardine flavour. Cream second grade.
22/11/37 ..	10 lb. mature and seedy	..	.. ..	Ditto.
23/11/37 ..	4 lb. fresh and green, but in seed	Morning milking	Two quarters milked at $\frac{1}{2}$ hr.	Distinct cress flavour. Poor first grade.
24/11/37 ..	1 lb. fresh and green, but in seed	Night milking	Two quarters milked at 1 hr.	Cress flavour slightly stronger.
26/11/37 ..	Ditto ..	..	1 hr. ..	Cress taint noticeable in hot cream.
			.. ..	Slight but definite cress taint in milk and cream. Cream first grade.

Juice from 1 lb. of land-cress was thus shown to be sufficient to cause land-cress taint in milk one hour after administration to a cow. The result may be compared with the findings of Babcock(10) that  $\frac{1}{2}$  lb. garlic, and of Eckles, Combs, and Derby(3) that less than  $\frac{1}{2}$  lb. of penny-cress, caused the milk to be tainted.

#### FEEDING OF LAND-CRESS PLANTS TO COWS IN STALLS

When cress was given to cows kept in stalls, it was noticed that only a portion was eaten. If the cress was mixed with a large proportion of Italian rye-grass, the cow was able to segregate and reject a proportion of the weed. Stall-feeding trials, depending on the voluntary consumption of cress by cows, are therefore liable to miscarry, and it was generally found preferable to use forced feeding. For this purpose the cress was minced and was then mixed with sufficient pollard and linseed-meal mixture to produce reasonably cohesive boluses, which were forcibly given to the cow at the selected time before milking. The results of some of these feeding trials are given in Tables II and III.

TABLE II.—RESULTS OF STALL-FEEDING TRIALS DEPENDING ON VOLUNTARY  
CONSUMPTION OF CRESS BY COWS

Date.	Details of Cress Feeding.	Interval between Feeding and Milking.	Remarks on Milk and Cream
15/9/39	One cow given 2 lb. of land-cress along with 20 lb. freshly picked Italian ryegrass	1 hr. ..	Cress taint detected in 0.4 per cent. concentration in fresh untainted cold milk. Detected in hot milk in 0.2 per cent. concentration.
16/9/39	Cow A : $\frac{1}{2}$ lb. cress given, only a portion consumed	„ ..	Cress taint in whole milk diluted 1:100 with clean milk.
	Cow B : $\frac{1}{2}$ lb. cress given, only a portion consumed	„ ..	Cress taint noted in the raw whole milk and in the cream diluted 1:20 with clean cream.
	Cow C : $\frac{1}{2}$ lb. cress given ..	„ ..	Cress taint noted in hot milk diluted 1:100 with clean milk. Strong cress taint noted in all three samples of morning's milk.
20/9/39	Cows Nos. 4 and 6 given 9 oz. cress mixed with 5 lb. grass. Only 2 oz. consumed by each cow	„ ..	Cress taint distinct in whole milks in concentrations of 1:50 in clean milk.

TABLE III.—RESULTS OF FEEDING TRIALS IN WHICH CRESS WAS FORCIBLY ADMINISTERED,  
MIXED WITH POLLARD AND LINSEED-MEAL

Date.	Details of Feeding	Interval between Feeding and Milking	Remarks on Milk, Cream, and Butter
25/9/39	Six cows, each given 1 lb. cress over period 1.30–2.30 p.m.; milked at 3 p.m.	$\frac{1}{2}$ –1 $\frac{1}{2}$ hr. . .	Cress taint in both night's and morning's creams; 14 lb. cream (whole of night's and morning's supply from the six cows) mixed with 970 lb. clean cream and made into butter. Distinct cress taint in flash-pasteurized cream and butter.
27/9/39	As for 25/9/39, but 2.2 lb. cress given to each cow at 12–1 p.m. and cows milked at 4 p.m.	3–4 hr. . .	Strong cress taint in night's milk and cream. Slight cress taint in morning's milk and cream. 13 lb. cream from these cows mixed with 900 lb. clean cream and made into butter. Strong cress taint in flash-pasteurized cream and butter.
3/10/39	As for 25/9/39, but 4 lb. cress given to each cow at 1–2 p.m. and cows milked at 3 p.m.	1–2 hr. . .	Very strong cress taint in night's cream. Faint cress taint in morning's cream. 4.5 lb. night's cream plus 10 lb. morning's cream with 950 lb. clean cream. Strong cress taint in mixed flash-pasteurized cream, and strong, very objectionable cress taint in the butter.

The results in Table II show that with the cows used, and in the early spring, only very small quantities of cress in the diet gave a cress taint in the cream which could be noticed in dilutions of 1 : 100 with good cream.

The results in Table III show that cress taint can be produced in cream by forced feeding of cress to cows, that it may persist even into the morning's milk and cream fifteen hours after administration of the cress, and that the taint was noticeable in the cream and butter when the combined night's and morning's creams were diluted with good cream in the ratio of 1 : 70.

The forced feeding method has been adopted as the best way of obtaining cress-tainted cream when required for buttermaking trials, and the above results have been generally confirmed, but the intensity of the taint obtained in the cream has shown wide variations for any fixed quantity of cress administered.

#### GRAZING TRIALS WITH SMALL GROUP OF COWS

The area used was a small paddock with grass 4-8 in. tall and badly infested with cress throughout. The cows were kept in the yard for some time prior to being turned on the cress area, in order to ensure that they would be sufficiently hungry to graze immediately. It was noted that they grazed both grass and cress, but they showed a preference for areas where the cress infestation was less dense, and in this respect there were individual-cow differences. Some of the cows were more selective in their choice of feed than others. None of the cows grazed on patches of cress not containing grass. The results for some of the trials are given in Table IV.

TABLE IV.—RESULTS OF GRAZING TRIALS WITH GROUP OF COWS ON SMALL CRESS-INFESTED AREA

Date.	Details of Time of Feeding.	Time Interval between Removal from Cress and Milking.	Remarks on Cream and Butter.
4/10/39	Six cows fasted 10.15 a.m.-12.15 p.m.; grazed on cress area 12.15-1.45 p.m.; milked at 3.15 p.m.	1½ hr. ..	Creams bulked. Cress taint in night's cream, not in morning's. Night's and morning's cream (16 lb.) mixed with 900 lb. of clean cream, flash-pasteurized. Slight cress taint in butter.
9/10/39	Six cows fasted 10.30 a.m.-1.15 p.m.; grazed on cress area 1.15-3.0 p.m.; milked at 3.15 p.m.	½ hr. ..	Creams bulked, Strong cress taint in night's cream, none in morning's cream. Portion of morning's cream flash-pasteurized and churned to butter in small churn. No cress taint in butter. Night's cream (10 lb.) in 900 lb. clean cream, flash-pasteurized, and made into butter. Strong cress taint in butter

#### GRAZING TRIALS WITH INDIVIDUAL COWS

The same cows as previously were used, but they were turned on to the area at different times, and the creams were kept separate. The separator bowl and the covers were washed between separation of each milk. In

some trials the creams obtained were mixed with fresh clean cream in the proportion of 1 : 4, and were made into butter after pasteurization to 190° F. in a small-model flash-pasteurizer. The results are given in Table V and Table VI.

TABLE V.—INDIVIDUALITY OF COW AND TIME OF FEEDING ON CRESS IN RELATION TO CRESS TAINT IN CREAM

Date and Series No.	Cow No.	Time Cow fasted.	Time on Cress Area.	Time of Milking.	Time Interval between Removal from Cress Area and Milking.	Intensity of Cress Taint (Range 0-10).	
						Cream	Butter
10/10/39 (Series I)	1	9.45-11.45	11.45-12.15	p.m.	Hr. min.	1	No cress.
	2	10.15-12.15	12.15-12.45	3.0	2.45	3	1
	3	10.45-12.45	12.45-1.15	3.0	2.15	3	No cress.
	4	11.15-1.15	1.15-1.45	3.0	1.45	1	0.5, slight cress.
	5	11.45-1.45	1.45-2.15	3.0	1.15	5	6, strong cress
	6	12.15-2.15	2.15-2.45	3.0	0.45	7	0.5, strong cress
14/10/39 (Series II)	1	9.30-11.30	12.0-12.30	12.30	0.00	0.5	Slight cress
	2	"	"	3.0	0.30	3	3; strong cress.
	3	"	"	1.0	0.30	3, strong cress	3; strong cress.
	4	"	"	3.0	2.0	3; strong cress	0, no cress.
	5	"	"	1.30	1.0	0; no cress	"
	6	"	"	2.0	1.30	1.5, cress	Strong cress
10/10/39 (Series III)	1	10.30-12.45	1.0-2.45	3.0	2.0	2; cress	Strong cress
	2	"	"	3.0	2.30	4; strong cress	Strong cress
	3	"	"	3.0	1.15	3	
	4	"	"	3.0	1.15	3	
	5	"	"	3.0	1.15	2	
	6	"	"	3.0	1.15	1	

#### COMMENTS ON RESULTS IN TABLE V

##### Series I

The gradings for cream and butter from cows 4 and 6 do not agree. Otherwise, the indications of the trial are that the more intense taint was obtained at the shorter period between ending of feeding and milking—viz., 15 minutes to 1 hour 15 minutes. The time between commencement of feeding and milking would be thirty minutes longer, as the cows were on the cress area for one half-hour.

##### Series II

The strongest taint was obtained with cow 6, when the interval after removal from the cress area was two hours and a half. Feeding of cow 1 on the cress area for thirty minutes, followed immediately by milking, did not give any cress taint in the cream, and only slight cress in the butter, but a second milking two hours and a half later gave strong cress in the cream. On the other hand, with cow 2 the cream from milk drawn one half-hour after removal from the cress area was as strongly tainted as the cream from the milk drawn two hours later.

##### Series III: Individuality of Cows

The six cows grazed on the area for the same time and milked at the same time gave differences in intensity of cress taint in the cream. In Series I and Series II trials the milk of cow 3 showed much less cress taint than that of the others, but this is not so apparent in Series III. It is not known how

much of this individual cow variation was due to variation in amount of cress consumed. But variations in individual cow reaction do occur, since they have frequently been noted for cows forcibly fed with the same amounts of minced cress. They may be due to variations in the period of retention of the cress plant in the rumen, or variations in the nature and volume of the contents of the rumen at the time the cress is consumed. It was noticed that after a period on a cress area or after being forcibly fed with cress the cows were eager to drink water. Trials were not made to investigate the relationship of amount of water taken after feeding of cress to intensity of cress taint in the milk.

#### FORCED FEEDING TRIALS WITH INDIVIDUAL COWS

Four cows were held on a cress-free paddock after the morning milking and were returned to the yard at 10.30 a.m. At 11 a.m. each cow was given freshly minced land-cress, and was then returned to the paddock. The cows were milked at 3 p.m. (evening milking), 8 p.m. (night milking), and at 5 a.m. (morning milking). The milks from each cow were weighed, and the creams from each cow were collected separately at each milking. Portion of each cream was examined after heating in a butter-moisture basin, and the three creams from the three milkings for each cow were then combined, flash-pasteurized at 190° F. in a small model flash-pasteurizer, and made into butter. The butters were examined for intensity of cress taint at the Dairy Research Institute and at the Auckland and Wellington Grading Stores. On the first day, 2 lb. of cress were fed to each cow, and on subsequent days 1 lb. only. The trial was carried out on four occasions over the period 1st to 6th November, 1943. The results are given in Table VI.

TABLE VI.—RELATIONSHIP OF INTENSITY OF CRESS TAIN IN CREAM AND BUTTER FROM COWS FORCIBLY FED THE SAME AMOUNT OF LAND-CRESS TO TIME INTERVAL BETWEEN FEEDING AND MILKING, TO INDIVIDUALITY OF COW, AND TO YIELD OF MILK (AVERAGE OF FOUR DAYS' TRIALS)

Cow.	Average Intensity of Cress Taint in Heated Cream (Scale 0-10).			Average Yield of Milk (pounds).			Average Intensity of Cress Taint in Butter (Scale 0-10).
	Evening.	Night.	Morning.	Evening.	Night.	Morning.	
A .. ..	3.75	5.5	1.25	9.2	6.5	8.5	4.6
B .. ..	4.5	5.5	2.5	16.5	9.1	16.3	3.2
C .. ..	3.2	4.5	0.5	12.7	7.0	11.5	2.4
D .. ..	3.9	5.25	0.25	10.2	7.3	16.1	1.9
Four cows	3.9	5.2	1.13	..	..	..	..

The creams and butters were graded for intensity of cress taint on the scale 0-10 as before. The figures for creams in the table represent the average of two independent gradings for each cream on each day—*i.e.*, eight gradings for each cow—and those for butter represent the average for the gradings at the Institute, at Auckland, and at Wellington.

#### (a) Time of Milking

It will be seen from Table VI that a very strong cress taint was present in the evening's cream four hours after the cress was given to the cows, and that it was still stronger at the night's milking, usually nine hours after

ingestion of the cress. Cress taint was present in the morning's cream from all the cows, though not consistently so—it was absent on 1 occasion out of 4 for cow A, 2 occasions out of 4 for cow C, and 2 occasions out of 4 for cow D. The persistence of cress taint over such a long period is unusual, but is of interest to the later discussion on controlled grazing as a means for suppression of land-cress taint.

#### *(b) Individuality of Cow*

Over the four days' average there was little difference in the intensities of cress taint recorded for the creams from the individual cows at the evening and night milkings, but a definite difference for the creams at the morning milking. The cress taint showed up much more strongly in the morning creams for cows A and B than for cows C and D. The figures for intensity of cress taint recorded for the butters reflect the effect of the admixture of the morning's cream, but are roughly in the order of magnitude of the intensity for the creams, although not in very close proportion.

#### *(c) Yield of Milk*

There was no indication of an inverse relationship between intensity of taint and yield of milk.

### INFLUENCE OF ADMIXTURES OF CLOVER AND LAND-CRESS

The possible effect of admixture of clover with land-cress fed to cows on the transmission of taint to the cream was investigated by forced feeding of 3 lb. of cress to cow E, and of a mixture of 3 lb. cress and 3 lb. clover to cows F and G. The following day the arrangement was reversed, cow E receiving the land-cress - clover mixture. There was no evidence from the examinations of either the creams or the butters that the strength of land-cress taint was related to the admixture of clover with the cress.

### SUMMARY OF TRIALS ON STALL FEEDING AND GRAZING WITH EXAMINATION OF MILKS OF INDIVIDUAL COWS

The conclusions to be drawn from the above results may be summarized as follows:—

- (a) The cress taint appears in the milk within half an hour of ingestion of cress. With some cows the taint is not completely eliminated in four hours, and when large quantities of cress are fed the cress taint may persist through to the next morning's milk and cream.
- (b) There are differences in intensity of cress taint in milks of individual cows fed the same quantity of cress or turned on to a cress area for the same period, but the individual cows are not consistent in their reaction to cress feeding.

### FEEDING OF LAND-CRESS HAY TO COWS

Many plants causing taint in milk retain their tainting properties when made into hay—e.g., alfalfa(13) and penny-cress(3). Land-cress was gathered in December, 1940, and was sun dried and then stored for four months. Two cows in stalls were given 10 lb. of land-cress hay. The



quantity remaining was collected and weighed after milking. The cream was separated, and was mixed with five parts of clean cream and was churned to butter. The results are given in Table VII.

TABLE VII.—QUALITY OF CREAM AND BUTTER FROM COWS GIVEN LAND-CRESS HAY

Date.	Cow No.	Quantity of Land Cress Hay consumed.	Interval Between Ingestion and Milking.	Quality of	
				Cream.	Butter.
10/4/40	A	1.0 lb.	1 hr.	No cress ..	No cress.
	B	4.5 lb.	..	Distinct cress	Slight cress.
13/4/0	B	5.5 lb.	1½ hr.	Cress taint ..	Slight cress.
	C	4.0 lb.	..	.. ..	No cress.

It is clear from the results in Table VII that the dried cress-plant will cause taint, but is less potent than the equivalent weight of the fresh plant.

#### FARM GRAZING TRIALS

##### (1) *Trials during December, 1939*

The first trials by the present authors were made during December, 1939, on farms at Tauranga and Te Puke. The cows were grazed on a cress-infested area all day on some days, and were removed at noon on other days during the period. The evening creams from the farm were graded by the factory-manager and the factory cream-grader on delivery at the factory next morning. The results are given in Table VIII.

TABLE VIII.—EFFECT OF CONTROLLED GRAZING ON GRADES OF CREAM FROM COWS ON CRESS-INFESTED PASTURE

Farm.	Date.	Number of Times Herd on Cress Area all Day.	Grade of Cream.	Number of Times Herd removed from Cress Area at Noon.	Grade of Cream.
A ..	6-17/12/39	7	Cress reported only on 2 days. Average grade, 91.4 (90-93)	5	No instance of cress. Average grade, 92 (91-93).
B ..	12-23/12/39	7	Strong cress flavour each time	5	Slight cress on one occasion.
C ..	11-24/12/39	4	Ditto .. ..	10	Strong cress on one occasion. Slight cress on one occasion.

Controlled grazing thus reduced the severity of the cress taint in the cream, but did not completely prevent the taint appearing.

##### (2) *Trials, September-October, 1940*

These trials were carried out on three farms, one supplying the Tauranga factory and two supplying the Te Puke factory:—

*Farm D.*—The infested area was a paddock of young grass 10-12 in. high, among which was a prolific growth of cress. There was no water available in the cress paddock. The evening milking began usually about 5 p.m. Portions of both night's and morning's cream from the herd were graded, and were then flash-pasteurized and made into butter. The butters

were graded by one of us (J. J. O'D.) and were then forwarded to Auckland for grading. The results are given in Table IX, and may be summarized as follows:—

TABLE IX.—RESULTS OF GRAZING TRIAL ON FARM D, TAURANGA

Date.	Time Cows removed from Area.	Grade of Night's Cream.	Grade of Butter.		Grade of Morning's Cream.	Grade of Butter.	
			D.R.I.	Auckland.		D.R.I.	Auckland.
Sept. 13-14	5 p.m.	Cress taint	Str. cress	Str. cress	No cress	No cress	? cress.
14-15	Midday	Sl. cress	Cressy and covy	V. str. cress	"	Slightly feedy; no cress	Covy.
15-16	5 p.m.	Cress taint	Str. cress	Str. cress	"	Fairly clean	Feedy.
16-17	Midday	Sl. cress	Feedy; sl. cress	"	"	Sl. cress	Feedy.
17-18	5 p.m.	Str. cress	V. str. cress	V. str. cress	? cress	Cressy	Str. cress.
18-19	Midday	"	Feedy and covy	Str. cress	Cress taint	Feedy; sl. cress	Str. feed.
19-20	5 p.m.	"	Str. cress	V. str. cress	Cress	Sl. cress; feedy..	Mod. cress.
20-21	Midday	"	Sl. cress	Str. cress	No cress	Feedy ..	Str. feed.
21-22	Clean paddocks	V. sl. cress	Feedy ..	V. little cress	"	Feedy and covy	Less feedy than night's.
22-23	all day	No cress	Str. feedy	Str. cress and covy	"	Fairly clean	Sl. covy and feedy.
23-24	Midday	No cress; feedy	Str. feedy and covy	Str. feedy; ? cress	"	Sl. feed	"
24-25	"	Sl. cress	Str. feedy and covy	V. feedy	"	No cress	"
25-26	"	"	Str. feedy	Cressy and feedy	"		
26-27	"	"	Str. feed; no cress	"	"		
27-28	"	No cress	Cressy and feedy	"	"		
28-29	Clean paddocks	"	? cressy	"	"		
	all day	"			"		

str. = strong, sl. = slight, v. = very.

Cows on cress all day (four times): Strong cress taint in night's cream and butter, and sometimes in morning's cream and butter. Cows removed from cress area at midday (eight times): Cress taint in night's cream or butter made from it on six days. Cress taint in morning's cream or butter on three days.

The trial was not a true trial of control grazing owing to the alternation between the two systems on the first eight days. The amount of cress taint in the cream during the four days 23rd to 27th September when control grazing was practised persistently was noticeably less, but it is not established that this was not due to some other factor, such as the cows becoming accustomed to dealing with the cress, although some farmers have stated that persistent grazing on cress does build up a reserve of tainting material in the system of the cow, and cress taint occurs in the cream for up to two days after the cows are removed from the infested area.

The owner of farm D reported that the trial had a noticeable effect on the health of the cows. They began to scour badly, they had a very strongly odorous breath, and the coat became rough and unhealthy in appearance.

*Trials on Farm E.*—The cress infestation was in old pastures about 6 in. in height, except for the two days 7th October, 1940, and 8th October, 1940, when the cows were turned on to an area of young grass with a heavy cress infestation, though not so prolific as at farm D.

In order to avoid the carry-over of the effect of all-day grazing of cress to a day of control grazing, as may have occurred in the trial at farm D, the cows were removed from the area at midday each day, right from the commencement of the trial. The results are given in Table X.

TABLE X.—RESULTS OF GRAZING TRIAL AT FARM E

Date.	Time Cows removed from Area.	Grade of Night's Cream.	Grade of Butter.	
			D.R.I.	Auckland.
2/10/40	11 a.m. ..	92; sl. feed; no cress	89; feedy; cressy	89; str. cowy.
3/10/40	12 a.m. ..	92; feedy; no cress	88; cressy and cowy	88; strong and cowy.
4/10/40	.. ..	92; no cress ..	87; str. cress ..	87; rank.
5/10/40	.. ..	90; sl. cress ..	87; unclean cress	86; objectionable unclean flavour.
6/10/40	5 p.m. ..	92; sl. feedy ..	89; no cress ..	87; rank.
	Young Grass Area.			
7/10/40	12 a.m. ..	88; str. cress ..	88; str. cress ..	87; rank.
8/10/40	5 p.m. ..	88; cressy ..	90; unclean feedy	90; str. cress.

The cress-infested pasture caused cressy taints in the cream and butter, but the cress taint was obscured to a greater extent by cowy and feedy taints. Feeding on the paddock of cress-infested young grass on 7th October, 1940, and 8th October, 1940, up till midday gave a strong taint in the night's cream.

*Trial on Farm F.*—The cress-infested area was a paddock seeded to grass after a maize crop. It was almost completely infested with cress although some camomile was also present. The plan of the trial was to turn the cows on to the area for an hour in the morning, and if cress was not found in the cream to bring forward the time of grazing on the area so as to shorten the interval between ingestion and milking time.

On 8th October, 1940, the cows were turned on to the area at 9 a.m. and were transferred to a clean area at 10 a.m. The night's cream was graded as 90, slight cress, and the butter was graded as 89½, cressy and feedy. The trial was not continued further.

ADDITIONAL EVIDENCE ON RELATION OF INGESTION OF CRESS TO CRESS  
TAINT IN CREAM

In addition to the results of the above systematic trials on the relation of cress ingestion to the appearance of land-cress taint in cream, a considerable volume of scattered data is available.

(a) *Cress Flavour in Large Volume of Cream when the Cress Infestation is Small*

Stirling(11) reports that "in one instance of pronounced land-cress taint in cream, search at the farm revealed the presence of only a few land-cress plants around gateways and fences adjacent to the milking-shed." Many such instances have been brought to our notice, as also of the effect of one small supply of cress-tainted cream causing cress taint in all the butter from a large vat of cream. The following are typical examples :—

*Factory A.*—Cress taint in the butter from 1,100 gallons of cream was traced to the cream from a "billy-can" supply drawn from one cow being grazed on a garden area. When this supply was rejected, the reduction in grade of butter due to cress taint ceased.

*Factory B.*—Cress taint was traced to one supply of 27 lb. cream from a small herd grazing on an old maize patch. This quantity of cream tainted the butter from a 2,000-gallon vat of cream.

*Factory C.*—The supply from two lame cows, turned on to a small area near the shed, where apparently they found some scattered cress-plants, caused strong cress taint in the cream from the remainder of the herd of 100 cows, and this cream in a 500-gallon vat of cream caused strong cress taint in the butter.

*Factory D.*—Ten pounds of cress cream from one supplier caused cress taint throughout thirty-seven boxes of butter.

The proportions of cress-tainted cream mixed with the cream supply in the above four instances were approximately : A, 1 : 3000 ; B, 1 : 740 ; C, 1 : 250 ; D, 1 : 407.

(b) *No Cress Flavour in Cream when Cows are grazing on an Area Heavily Infested with Cress*

Stirling(11) reports also that "at another farm in the same district all the pastures were known to be heavily infested with cress, and numerous inspections showed that the plant was heavily grazed by the herd, yet careful daily examinations of the cream revealed the typical flavour on one occasion only."

During the season 1943-44 on the Massey Agricultural College Farm the herd each week-end from 13th September to 2nd November was turned on to an area of young grass heavily infested with cress, with the object of obtaining a supply of cress-tainted cream for cream-processing trials in the factory. The herd was left on the area each day until milking-time, and the cows were observed to be eating the cress, but cress taint was not found in the herd creams. Forced feeding of cress-plants collected from the area resulted in the production of cream strongly tainted with cress.

Instances of ingestion by cows of land-cress plants without the appearance of cress taint in the cream are more frequent in the late spring and early summer than in the early spring months. This is not due to a difference in content of mustard-oil glucoside, since it has been found(1) that there is little change in glucoside content during the season from September to

February, and the cress collected from the Massey Agricultural College grazing area referred to above had a normal content of the mustard-oil glucoside. In normal years cress taint is not frequently troublesome after the middle of December, but in years of persistent rainfall, such as the season 1945-46 in the Manawatu district, the trouble may persist into February. A dry autumn, followed by warm rains bringing on a flush of young grass, may be accompanied by a return of trouble due to cress taint in the butter.

(c) *Season of Year and Appearance of Cress Taint*

As stated earlier, occurrences of cress taint in cream and butter are more frequent in spring than in summer and autumn. This is to be expected, because the growth of the plant is more prolific during the spring. Many instances can be quoted, however, where cress taint ceased to occur before the cress infestation had disappeared from the pasture. In some feeding experiments at the Institute during a dry February it was found that 6 lb. of cress could be given to a cow one to two hours before milking without causing any marked cress taint in the cream, and it was established that the plants contained a normal amount of mustard-oil glucoside. It is not established whether the relatively small effect of the plants in the autumn is due to any change in the plant itself, or whether it is due to an increase in the ability of the cow to throw off the tainting substance.

(d) *Results of Controlled Grazing for Suppression of Cress Taint in Farming Practice*

Following the work described above on controlled grazing, a number of factory-managers, with the support of their directorates, adopted the principle of rejecting cress-tainted cream (all suspected cream samples were graded hot), and circularized the suppliers advising them to remove cows from the cress-infested pastures at least three hours before milking. The results have been fairly satisfactory. Controlled grazing was practised regularly during the season 1940-41 at farms B and C referred to above, and no instances of cress taint in the cream from these farms were reported. On one day at farm B in the following year the cows were inadvertently left on a cress area all day. The owner noticed the cress taint in the milk and cream soon after milking was commenced. The milking was then interrupted for two hours, and the two lots of cream were kept separate. The cream from the cows milked before the interruption was rejected at the factory, but that from the cows milked after the interruption was accepted as free from taint.

Reports from factories where suppliers have been practising controlled grazing indicate that the system gives a very marked improvement in the cream-supply, but it is not completely effective, especially where the cress infestation is dense. This is supported by the results given above of the feeding trials with individual cows, in which cress taint persisted into the morning milking fifteen to sixteen hours after ingestion of the cress. These results, together with those for the trials on farms A and D, in which the evening's and sometimes the morning's cream were tainted, and those for the trials on farm E, in which the evening's cream was tainted although the cows were grazed on the cress area only from 9 a.m. to 10 a.m., demonstrate clearly that the system of controlled grazing for cress taint, like that for clover taint (Davies(5), Marryat(6)) is a palliative, but not a sure corrective. It should be adopted only if it is not practicable to adopt more positive alternative methods, such as grazing the infested area with dry stock.

## CRESS INFESTATION IN RELATION TO FARMING PRACTICE

(a) *Factors favouring the Appearance of Cress in a Pasture* (Allo(12) )

Cress infestation of a pasture may take place from one or more of the following causes :—

- (1) Heavy stocking or trampling of a pasture during winter, with consequent over-grazing and pugging, leading to a deterioration and opening up of the sward.
- (2) The practice of leaving old stack bottoms and silage pits unfenced from stock. Cress grows freely in such places, and may cause trouble, even though the areas are small. All such areas should be shut off from stock until the end of December.
- (3) The occurrence of bare ground due to heavy tramping around gateways, cowyards, and stockyards, and, due to shade, under hedges and around the base of thistles, gives conditions where cress is likely to appear and thrive. It is not always possible to fence off these areas, but they should be watched and any cress-plants destroyed by hand cultivation.
- (4) Failure to chain harrow sufficiently frequently, which may allow the growth of cress around old cowpads.
- (5) Grass-grub attack, which weakens the pasture growth and leaves bare patches on the sward.
- (6) Leaving paddocks uncultivated after a crop such as maize or turnips. These paddocks should not be grazed by milking-cows.
- (7) Spring sowing of pastures, especially after a maize crop. The danger is accentuated if cheap seed is sown or if the mixture of seed contains a high proportion of slow-establishing pasture species.

(b) *Prevention of Cress Infestation*

From the list of factors favouring cress infestation, as cited above, a number of necessary corrective measures can be deduced. These may not always be applicable, as, for example, the prevention of pugging around stacks and cowyards during a wet season. When this applies, the weed should be prevented from growing by cultivation or use of weed-killers, or the cows should be kept away from the area. Besides these factors, which may affect only small areas, the most important preventive measure is to *avoid spring sowing of pastures whenever possible, and avoid the use of poor mixtures of pasture seed. The pastures should be sown in the early autumn on a well-prepared seed-bed, with adequate phosphate manuring to bring the young grass quickly into growth.* A young pasture that is well developed before the winter check to growth occurs will have few bare areas where cress seeds can germinate and grow in the spring. If spring sowing cannot be avoided, the mixture should be mainly rye-grass and white clover, to provide a quick cover and to prevent germination of cress seeds.

(c) *Grazing of Cress-infested Pastures*

These should be grazed with dry stock, if this is possible without reducing the food-supply too much for the milking-herd. If it is necessary to graze an infested pasture, the time of grazing in relation to time of milking should be carefully controlled, and the time interval should be at least three hours, but preferably four to five hours. There is a general belief among farmers in districts where cress trouble is prevalent that feeding of hay during the

period after removal from the cress area is an advantage, and on first principles this seems likely to be correct, but it has not been checked by experimental trials. If the growth of grass is ample, there is considerable difficulty in inducing the cows to eat hay. Good green hay is more likely to be eaten than a poor-quality fibrous hay.

(d) *Distribution of Cress Plants*

Random cress-plants may be destroyed by hand cultivation (since land-cress hay also can cause taint, the cut plants should be removed out of reach of the milking-herd) or by spraying with a suitable weed-killer.

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(To be continued)

## METHODS OF MEASURING THE PRODUCTION FROM GRASSLANDS

### A REVIEW OF THE TECHNIQUES EMPLOYED BY THE FIELDS DIVISION, DEPARTMENT OF AGRICULTURE

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#### *Summary*

The paper summarizes techniques which have been employed by the Fields Division of the Department of Agriculture for measuring grassland production. The development of these methods is indicated from early haying and grazing trials to techniques such as that involving the use of "pure clover swards" which are being developed and investigated at the present time. Hudson's "mowing and grazing technique" is examined in some detail, particularly with reference to the effect of "transference of fertility," which has been stated to affect the accuracy of data secured under this technique. Results are presented which indicate the extent of "transference" that is likely to occur under normal trial conditions. Some suggestions for improvements in existing techniques and possible methods worthy of investigation are also included.

The paper shows the necessity of developing special techniques for special purposes and suggests possible stages in the experimental approach to grassland problems. For small-plot trials "mowing and grazing" trials, "mowing only and clippings returned" technique, "Sear's" technique, and the "pure clover sward" technique may be used; for large-scale trials the "enclosure" technique, which involves the use of "frames" or "enclosures" in paddocks under grazing, whereby areas are protected for herbage production figures, has proved most satisfactory. The use of demonstrational and observational trials on farmers' properties is considered the final stage in an investigation of the type discussed.

THE measurement of grassland production is one of the most difficult problems the experimentalist has to overcome, particularly where it is necessary to measure relatively small differences between fertilizer treatments or improved pasture species. The problem is further complicated by the fact that it is desirable, where practicable, to measure such differences in terms of stock-carrying capacity and production. The intimate relationship between the sward and the grazing animal cannot be overlooked, for the animal is a major factor in determining the production from pastures. More particularly, however, the kind of stock used and the severity of grazing profoundly influence the pasture species that make up the sward. Each pasture element reacts differently to grazing and management, and on an experimental scale, at least, it is possible to vary the sward composition at will by adjusting the management to which the pasture is subjected. The effect of this factor in experimental work can be readily appreciated.



## HISTORY OF THE METHODS EMPLOYED

(1) *Haying Experiments*

Before the introduction of the "mowing and grazing technique" (1) this was the most commonly used method. It was discontinued owing to the defects in technique, which may be summarized as follows:—

- (a) The measurement is of production over a portion of the year only and takes no account of critical periods of low pasture production.
- (b) The trial cannot be satisfactorily continued for more than one year owing to the deterioration of the sward resulting from successive hay crops.
- (c) The measure is a composite one over a long period of growth and can give no indications of variations in production within this period.

(2) *Grazing Trials*

These trials were first carried out under a simple design and technique requiring the determining of the number of stock-grazing days which paddocks of several acres were able to support. Such trials are mainly demonstrational rather than experimental, for the size of the individual plot makes adequate replication of treatments impossible. In most cases it is not possible to eliminate an appreciable part of the errors associated with stock-grazing trials, such as the relative efficiency of utilization of the herbage. Although some improvement was effected by considering stock weights, the efficiency of these trials is much lower and the cost much higher than that of trials which have as their object the measurement of total grassland production. With such experiments one treats as a separate problem the relation of production data to stock-carrying capacity and stock-production figures.

## HERBAGE WEIGHTS IN RELATION TO ANIMAL PRODUCTION RECORDS

There is no question, however, of the desirability of measuring grassland production in terms of animal production, and the most recent efforts to improve technique have been directed along these lines overseas (2, 3).

In New Zealand the Fields Division has conducted investigations into technique with this end in view for many years, and these investigations will be summarized later in this article. Mr. P. D. Sears (4), of the Grasslands Division, Department of Scientific and Industrial Research, has made a valuable and original contribution to the study of the interaction between the sward and the grazing animal, and his technique will be discussed in some detail. However, once the grazing animal is used as the yardstick of measuring differences due to treatment, small-scale plot work is out of the question. Furthermore, the large-scale, expensive, and laborious stock-grazing trial cannot be adequately replicated from a statistical point of view if many treatments are involved. One is thus forced to the following method of approach:—

- (a) Preliminary work with many treatments using small-scale plot trials.
- (b) Large-scale animal plus grassland production trials for important investigations arising out of (a) above.
- (c) Demonstrational simple stock-grazing trials and observational trials conducted over a wide range of soils and climates to extend the results from (a) and (b) to the farming community as a whole, including the use of simple methods of measuring grassland production to amplify and check these trials wherever possible.

The Fields Division is in the fortunate and possibly unique position of being able to carry investigations through all these stages. The phases (a) and (b) above are carried out on experimental areas, and it is hoped that the new Soil Fertility Research Station at Hamilton will make a valuable contribution in this respect. The extension and amplification of results from the experiment station to the farmer under (c) above is carried out by means of co-operative trials on farms, and has proved, in many instances, an essential part of the research programme as well as an efficient and rapid means of making known new findings to the farming community.

The foregoing will have served to illustrate the need for finding the most suitable technique to apply to each of the above types of trials.

#### A. SMALL-SCALE PLOT TRIALS INVOLVING MANY TREATMENTS AND REQUIRING MEASUREMENT OF GRASSLAND PRODUCTION

These may be considered as follows :—

- (1) "Mowing only" trials.
- (2) "Mowing and grazing" trials (Hudson *et al*(1)).
- (3) "Mowing only and returning clippings" technique.
- (4) Technique of P. D. Sears(4) involving collection of sheep faeces and urine.
- (5) "Pure white clover sward" technique.
- (6) Additional methods worthy of trial.

##### (1) "*Mowing only*" and *discarding Clippings*

In all the methods to be described (except (6)), a motor lawn-mower has been used as providing the most efficient means of obtaining pasture-production data. Hudson(1) soon found that the effect of continuous use of the lawn-mower alone was rapidly to produce a sward which differed in botanical composition from a comparable pasture under sheep-grazing. Low-growing elements such as "flat" weeds and clovers were encouraged at the expense of taller-growing grass species, and the sward and its production rapidly deteriorated. This deterioration is hastened by the removal of nutrients in the form of grass clippings. For these reasons, endeavours have been made in methods (2) and (4) below to (a) introduce the selective element implied in sheep grazing, and (b) return the nutrients removed in the grass clippings.

##### (2) "*Alternate Mowing and Grazing Technique*" (Hudson(5))

The first great advance in the improvement of technique was A. W. Hudson's "alternate mowing and grazing" method of measuring pasture production. Briefly, this comprises two duplicate sections (A and B) in each of which the treatments are arranged in a series of "randomized

blocks" (or "latin squares"). Two types of mowing are made: (a) "M and W," when herbage is mown and weighed; and (b) "M and C," when herbage is mown to clear up growth to an even "mower height" before leaving to grow for a "M and W" cut. The sequence is as follows:—

Day.	Section A.	Section B.
1	Closed .. .. .	Closed.
6	.. .. .	Grazed.
11	.. .. .	Grazed.
12	M and W (1); herbage transferred to Section B	Grazed.
24	M and W (2); herbage left on plots from which cut; section grazed	M and C; clippings transferred to Section A.
25	Grazed .. .. .	..
30	Grazed .. .. .	..
36	Grazed .. .. .	M and W (1); herbage transferred to Section A.
37	Grazed .. .. .	..
48	M and C; clippings transferred to Section B	M and W (2); herbage left on plots; section grazed.

By this means continuous production records are secured, while each section alternates between sheep-grazing and mowing and, over a period, receives back through the sheep the nutrients it has produced. Furthermore, the selective effect of the grazing animal is introduced to maintain a typical sward under grazing conditions.

A high degree of accuracy of measurement is possible with such a trial and very satisfactorily low standard errors are associated with the results, as Table I illustrates.

TABLE I.—STANDARD ERRORS OF MOWING AND GRAZING TRIALS

Trial.	Standard Error per Plot as a Percentage of Mean Plot Yield.
Marton I, 1941-42 .. .. .	1.3
" 1942-43 .. .. .	1.4
" 1943-44 .. .. .	1.0
Marton N, 1940-41 .. .. .	1.5
Marton G, 1932-40 (eight years) .. .. .	1.4
Marton H, 1932-37 (five years) .. .. .	1.0
Ruakura A, 1936-39 (three years) .. .. .	1.5
Ruakura B, 1936-39 (three years) .. .. .	1.2
Ruakura C, 1936-39 (three years) .. .. .	1.4
Ruakura F (a) 1940-41 .. .. .	1.8

Unfortunately, however, the "mowing and grazing technique" suffers from two serious defects:—

- (1) The sward which is formed is well-nigh perfect from the investigator's point of view, but practically unobtainable by the farmer. The form of management allows of no growth beyond a leafy 4 in. to 6 in., and this, while ideal for the highly productive sward, is impossible of realization by the average farmer. The technique does, however, measure the *maximum* which a sward may be expected to produce under the seasonal conditions prevailing.

- (2) As stock graze all treatments within the one enclosure and their droppings are distributed more or less uniformly over all, it is to be expected that nutrients will be transferred by this means from the highly productive plots to those of lower yield.

This transference of fertility was fully recognized by Hudson(1). Sears(4) makes its elimination the main aim of his elaborate technique. Further, a special trial to endeavour to measure this "transference" was carried out by the Fields Division at the Ruakura Animal Research Station, Hamilton. Before considering this experiment it is of interest to examine the responses from trial G at Marton over a period of eight years and the relative production from the control plots (no treatment) (Table II). Results from this trial have been published in this *Journal*(6).

TABLE II.—" TRANSFERENCE OF FERTILITY " ON MOWING TRIAL G, MARTON

Period.	Number of Days.	Dry Matter, in Pounds per Acre.	
		Yield of Control adjusted to three hundred and sixty-five Days.	Difference between Control and Treatment 5.*
5/10/32 to 2/10/33 .. ..	362	8,720.0	13.3 (100.2)†
2/10/33 to 3/10/34 .. ..	366	8,469.7	598.8 (107.1)
3/10/34 to 26/9/35 .. ..	348	7,748.8	217.7 (102.8)
26/9/35 to 29/9/36 .. ..	369	12,487.6	339.4 (102.7)
29/9/36 to 5/10/37 .. ..	371	12,055.5	646.2 (105.4)
5/10/37 to 3/10/38 .. ..	363	8,268.8	464.0 (105.6)
3/10/38 to 2/10/39 .. ..	364	6,945.8	1,029.1 (114.8)
2/10/39 to 27/8/40 .. ..	330	8,803.4	900.0 (110.2)

\* Treatment 5 = carbonate of lime 5 cwt. each year.  
relative to control as 100.

† Figures in parentheses are yields relative to control as 100.

There is not the consistent increase in the yield of the control plots that one would expect from "transference of fertility" (in this case of calcium, as all the treatments except control were lime treatments). It is admitted, however, that the figures do show such marked seasonal variations that any "transference" would not be readily apparent. There is no evidence of any loss of measurable response in the relative yields of treatment 5 in the last two years of the trial.

Three "mowing and grazing" trials (A, B, and C) at the Ruakura Animal Research Station, Hamilton, were laid down in the autumn of 1938 on each of three differing soil types. The treatments for each trial were:—

- (O) (1) Control.  
 (L) (2) Carbonate of lime 1 ton initially plus 5 cwt. annually.  
 (P) (3) Superphosphate 3 cwt. annually.  
 (PL) (4) Superphosphate 3 cwt. annually plus carbonate of lime as in (2).  
 (PK) (5) Superphosphate 3 cwt. plus 30 per cent. potash salts 2 cwt. annually.  
 (LPK) (6) Superphosphate 3 cwt. plus 30 per cent. potash salts 2 cwt. annually plus carbonate of lime as in (2).

Until the winter of 1939 these trials were under "alternate mowing and grazing" technique, and from that period till March, 1942, under "mowing only and clippings returned" technique (see Section (3) below), where no "transference of fertility" is possible and the trials are not grazed. Table III summarizes the results from these trials.

TABLE III.—RESULTS FROM TRIALS A, B, AND C, RUAKURA

Treatments.	Relative Yields of Dry Matter.	
	"Alternate Mowing and Grazing" (1936-39).	"Mowing only" (1940-42).
Trial A—		
(1) (control) .. .. .	100	100
(2) L .. .. .	102.7	99.4
(3) P .. .. .	114.5	119.4
(4) LP .. .. .	118.6	122.8
(5) PK .. .. .	117.3	125.4
(6) LPK .. .. .	122.7	129.8
Trial B—		
(1) (control) .. .. .	100	100
(2) L .. .. .	106.1	108.4
(3) P .. .. .	105.4	109.0
(4) LP .. .. .	111.1	114.5
(5) PK .. .. .	106.7	110.9
(6) LPK .. .. .	112.0	116.3
Trial C—		
(1) (control) .. .. .	100	100
(2) L .. .. .	106.3	103.8
(3) P .. .. .	118.1	125.6
(4) LP .. .. .	125.1	132.3
(5) PK .. .. .	118.8	128.1
(6) LPK .. .. .	126.3	132.1

Table III indicates a greater measured response from all treatments except "lime alone" (treatment 2) under the technique where transference of fertility is eliminated. This suggests that "transferred" phosphorous and potash are more effective than calcium in obscuring differences between treatments in trials under "mowing and grazing" technique. If nitrogen, as widely believed, is the most effective nutrient transferred by stock, it is difficult to account for the lack of increase of responses to treatment 2 as shown in Table III on trials A and C and the lack of evidence for "transference" in Table II. The suggestion that "returned" clippings are picked up in the next mowing and weighing and erroneously increase the response is not supported by the reaction of the "lime alone" plots (treatment 2 in Table III) under such a technique on trials A and C.

An attempt to measure the amount of transference of fertility was made at Ruakura. Mowing trial F was under action from October, 1938, to February, 1943. The layout of this trial was in two sections, as illustrated in Fig. 1. The central portion of the trial was under the usual "mowing and grazing" technique with six treatments each replicated six times in

each of two enclosures. The treatments were identical with those of mowing trials A, B, and C at Ruakura (see before). Two of these treatments (control (A) and superphosphate plus carbonate of lime (B)) are included in the outer section of the trial. Here each treatment is in a separate enclosure, so that the question of transferred fertility does not arise. These treatments are replicated in pairs four times, each pair being treated as a separate mowing and grazing trial, but with only one treatment involved.

A <sub>2</sub>	A <sub>1</sub>		B <sub>8</sub>	B <sub>7</sub>
B <sub>1</sub>	1	5	3	A <sub>8</sub>
	2	6	4	
	3	1	5	
	4	2	6	
	5	3	1	
	6	4	2	
B <sub>2</sub>	1	5	3	A <sub>7</sub>
	2	6	4	
	3	1	5	
	4	2	6	
	5	3	1	
	6	4	2	
A <sub>3</sub>	4	2	6	B <sub>6</sub>
	3	1	5	
	2	6	4	
	1	5	3	
	6	4	2	
	5	3	1	
A <sub>4</sub>	4	2	6	B <sub>5</sub>
	3	1	5	
	2	6	4	
	1	5	3	
	6	4	2	
	5	3	1	
B <sub>3</sub>	B <sub>4</sub>		A <sub>5</sub>	A <sub>6</sub>

FIG. 1.

While one member of the pair—e.g., A<sub>1</sub>—was under mowing, the other member (A<sub>2</sub>) was under grazing, and mowings and grazings alternated as in the central trial, though not necessarily at the same dates. The soil type was that of trial A above, a Horotiu sandy loam. (It will be noted that the layout is on a "balanced" rather than a random design, as were all earlier trials. To this extent the statistical analysis of the trial may be considered invalid, but with the large number of replications of treatments it is most unlikely that the results are unduly biased.)

Table IV summarizes the results from trial F, and, for comparison, comparable figures are given for trial A. Total production figures are not given, because during the war staff difficulties resulted in two gaps in the records, each of about one month.

TABLE IV.—RESULTS FROM TRIAL F, RUAKURA, 1938-43

Treatment.	Relative Yields of Dry Matter.			
	Trial F.		Trial A.	
	Usual "Mowing and Grazing."	"Mowing and Grazing" in Separate Enclosures.	Usual "Mowing and Grazing" (1936-39).	"Mowing only" (1940-42).
(1) Control ..	100	100	100	100
(2) L ..	105.0	..	102.7	99.4
(3) P ..	117.5	..	114.5	119.4
(4) LP ..	123.0	130.9	118.6	122.8
(5) PK ..	122.7	..	117.3	125.4
(6) LPK ..	127.0	..	122.7	129.8

Considering Table IV, it would seem that there has been a loss of measured response on the LP plots due to transference of fertility of 7.9 per cent. in trial F (1938-43) and 4.2 per cent. in trial A. The layout of trial F does not permit the statistical examination of this difference, but on trials A, B, and C the interaction of manures  $\times$  techniques (based on green weights) is of a much smaller order than the "interaction" manures  $\times$  seasons. This implies that the difference in technique does not affect the response to treatments.

In an unpublished paper (1937), B. W. Doak, Chemist, Grasslands Division, Palmerston North, has examined "transference of fertility" from the chemical viewpoint. He states that—

... It will probably be admitted that on such a phosphate-responsive soil as exists at Marton that any transference of phosphoric acid from the phosphated to the non-phosphated plots may cause an increase in growth. However, on looking into the facts of the case we find the available  $P_2O_5$  of the soil from trial I as follows:—

AVAILABLE  $P_2O_5$  OF SOIL OF TRIAL I, MARTON  
(Depth of Sampling 0-2 in.)

	Per Cent.
Average of whole area at commencement of experiment, 1932	0.018
Headland (unphosphated), 1937 .. ..	0.012
Superphosphate (treatment 1), 1937 .. ..	0.035
Basic slag (treatment 2), 1937 .. ..	0.033
North African phosphate (treatment 3), 1937 .. ..	0.036
Superphosphate plus lime (treatment 1L), 1937 .. ..	0.041
Basic slag plus lime (treatment 2L), 1937 .. ..	0.038
North African phosphate plus lime (treatment 3L), 1937 .. ..	0.045

(Phosphate applications were annual dressings of 224 lb. superphosphate, 306 lb. basic slag, and 175 lb. North African phosphate per acre (equal amounts of total  $P_2O_5$ ). The lime applications were 1 ton per acre carbonate of lime initial dressing and 3 cwt. per acre per annum subsequent dressings.)

A consideration of these figures shows that, in spite of the build-up of 100 per cent. in available  $P_2O_5$  percentage of the top 2 in. of the soil as the result of phosphate treatment in five years, there has been a drop of 33 per cent. in the available  $P_2O_5$  of the headland (unphosphated) during the same time. The result is that after five years the available  $P_2O_5$  of the headland is less than one-third of that of the phosphated area. Surely this does not indicate any transference of phosphate as the result of grazing sheep.

If the figures for lime are considered, a similar state of affairs is found to exist. Considering the exchangeable  $CaO$ , we find that in 1932 the percentage exchangeable lime was 0.192 per cent. In 1937 the untreated headland had been reduced in exchangeable lime to 0.177 per cent., in spite of the fact that the various treated plots in the same enclosure have risen to 0.261, 0.324, 0.246, 0.376, 0.431, and 0.371 per cent., for the treatments 1, 2, 3, 1L, 2L, and 3L respectively.

Having considered the status of the soil with respect to two of the major mineral constituents, we will now consider the nitrogen status. This shows a very different state of affairs. In 1932 the percentage of total nitrogen of the soil was 0.29 per cent., whereas by 1937 this figure had risen to 0.39 per cent., an increase of nearly 35 per cent. On first sight this might appear a strong argument for the "transference of fertility" case, but I am unable to agree with this contention, as we have found that the total nitrogen percentage of the soil from just over the fence has risen to 0.37 per cent. This field has not been so heavily fertilized, nor has any portion of it been differentially treated, so that the build-up in total nitrogen in this instance cannot be ascribed to transference of fertility. It must be attributed to a combination of the natural build-up in total nitrogen by soil organisms, the decay of the lower leaves of the grasses, and of the effect of the increased stock nitrogen returned to the soil as a result of some fertilizer treatment associated with the type of management practised.

In the differentially treated enclosure there is some variation in the total nitrogen content of the various treatments, though there is no reasonable correlation with the total herbage production, and it would appear that the various phosphate treatments alone and in association with lime are affecting the availability of the total nitrogen. On the limed areas the availability of the nitrogen would appear to be greater than on the unlimed.

Summing up . . . the inaccuracies which may be associated with the mowing and grazing technique must be due to the fact that an endeavour is being made to measure a fertilizer response while the total nitrogen content of the soil is continuously increasing . . .

It should be realized that the Marton trials under consideration were put down on young pasture. As a result of cultivation the organic matter would have been distributed in the cultivated layer and there would be no accumulation in the top layer . . .

The foregoing material has shown that the effect of transference of fertility is to diminish the measurable response from treatments to a real extent, though not to the extent thought possible by other investigators. Further, the responses measured by the mowing and grazing trial are real, though conservative, differences. Nevertheless, it is desirable to find a means of eliminating this source of error, and the following techniques are designed with this in view.

### (3) "*Mowing only and returning Clippings*" Technique

This method has been used on trials A, B, and C at Ruakura (see above) and also on trial P at the Marton Experimental Area. Essentially the technique consists in returning the herbage that has been produced to the plots after mowing and weighing. The trial area is not grazed and thus no



transference of fertility is possible. The clippings are scattered over the plots from which they were mown. The two main defects of this method of measuring pasture production are :—

(a) The selective element of sheep grazing is not introduced, and after a time (though not as quickly as when the clippings are discarded) the close-growing elements of the sward, such as the clovers and "flat" weeds, are encouraged at the expense of the taller growing grasses. Production as measured by mowing slowly falls off because of the low-growing habit and lowered relative production of the resulting sward. Table V should be examined in relation to this.

TABLE V.—PRODUCTION UNDER "MOWING AND CLIPPINGS RETURNED" COMPARED WITH PRODUCTION UNDER "MOWING AND GRAZING": MARTON EXPERIMENTAL AREA

Dry matter in pounds per acre (totals adjusted to three hundred and sixty-five days)

Year.	Trial P (Mowing only).		Trial I (Mowing and Grazing).	Difference.	
	Control (no Treatment) (a).	Superphosphate 3 cwt. per Acre per Annum (b).	Superphosphate 3 cwt. till 3rd April, 1940: none subsequently (c).	(c) - (a)	(c) - (b).
September, 1939, to September, 1940	8,040.4	9,399.2	9,546.5	+ 1,506.1	+ 147.3
September, 1940, to September, 1941	8,580.8	10,063.0	8,349.0	- 131.8	- 1,714.0
September, 1941, to September, 1942	11,088.1	11,841.9	11,253.8	+ 165.7	- 588.1
September, 1942, to September, 1943	5,952.8	6,027.0	6,082.6	+ 129.8	- 844.4
September, 1943, to September, 1944	7,516.6	8,828.5	8,149.8	+ 633.2	- 678.7

At Marton, considering the difference (c) - (a) after the first year, when the residual affect of the superphosphate dressing is comparatively small, there is a slow decline in production of the "mowing only" control plots compared with those of the "mowing and grazing." Superphosphate (c) - (b) has more than maintained production on trial P. Nevertheless, this production was largely secured from inferior species, notably *Poa trivialis*, and the sward was showing rapid signs of deterioration when the experiment was closed.

At Ruakura, on the other hand, the composition of the sward was relatively unchanged in the two years of trial under "mowing only." Production data, unfortunately, do not allow of comparison between this and the "mowing and grazing" technique, due to incomplete records owing to staff difficulties during the war.

However, a technique trial was carried out at Ruakura during the years 1937-39 in which the following treatments were tried :—

- (a) "Mowing and clippings returned."
- (b) "Mowing and clippings removed."
- (c) Mown twice as often as (a) and (b), and clippings returned.

Table VI summarizes the results from this trial.

TABLE VI.—YIELDS OF GREEN MATERIAL, IN POUNDS PER ACRE, FROM PASTURE AS MEASURED BY THREE TECHNIQUES

Period.	Technique (a).	Technique (b).	Technique (c).	Yields relative to (a) as 100.	
				Technique (b).	Technique (c).
17/9/37 to 29/8/38 ..	54,886.3	41,500.3	49,320.6	75.6	89.9
29/8/38 to 1/9/39 ..	53,406.1	33,671.3	50,923.7	63.0	95.4
Total period	108,292.4	75,171.6	100,244.3	69.4	92.6

On only two occasions was there any sign on series (a) of clippings from the previous cut when the area was about to be mown and weighed. The sward on series (c) contained a higher proportion of clovers than on series (a), but the latter sward showed better recovery after cutting. The sward on series (b) deteriorated rapidly and showed poor recovery after cutting. Compared with an adjacent sward under grazing, it was observed that if the clippings were returned after mowing there was less difference between mowing only and grazing only than between one type of grazing and another.

(b) The other defect of this technique is that, at certain seasons of the year, some of the clippings from the previous weighing may be picked up and reweighed with the fresh growth. This would falsely increase the figures both for total production and for the response to treatments. Table V and Table III show that this effect is not marked when yearly production and total response data are examined. The relative yields from the "lime alone" plots in trials A and C, shown on Table III, indicate that this factor cannot be of great importance.

Nevertheless, at certain times cutting is followed by three or four days of dry, warm weather, and under these conditions some of the dried clippings may be seen at the next mowing. Their total amount is small, but some endeavour must be made to remove and discard them before mowing and weighing. In other circumstances, and in the majority of weighings, no trace of the clippings remain at the following cut. Cuttings must, however, be made at more frequent intervals and shorter stages of growth than is possible with trials under "mowing and grazing."

At present it would seem that, providing a close watch is kept on the sward for possible deterioration, this technique, because of its ease of operation, will be a useful means of making preliminary comparisons between treatments. It is probable that its use will have to be restricted to districts of higher rainfall where in the majority of cases the clippings will have decomposed sufficiently and have been removed to below the level of cutting before the next mowing. Care will also have to be taken to see that the plots are well sheltered, to prevent, as far as possible, the removal of clippings by wind.

(4) "*Sears*" Technique: *Return of Stock Droppings in Proportion to Treatment Production*

This technique has been fully described by Sears(4). Briefly, it involves the following :—

- (a) The plots are mown, herbage weighed, and replaced on the plots from which it was cut. Samples of green herbage are taken for dry-matter determinations and botanical analysis.
- (b) Sheep are grazed on the area containing the plots in sufficient numbers to clean the growth down to mower height in two to three days. Thus the amount of herbage yield is short of what is actually grown by the total of growth over the grazing days. These sheep are fitted with collecting bags and bottles(7). The total collection is mixed in a barrel, sieved, and measured. This total mixture is then watered back on to the plots with a special watering-can in proportion to the amount of dry matter yielded by each plot (and headland). The dry-matter determinations are carried out whilst the excrement is collected in grazing.

This technique overcomes "transference of fertility" while at the same time retaining the element of selective grazing by stock. On the results obtained using the technique, doubt is thrown on those from "mowing and grazing" trials. However, the present writer claims that the comparative figures quoted cover too short a period and are based on data which cannot be treated statistically to warrant such a conclusion. Although the Fields Division has not, as yet, carried out trials under the "Sears" technique, experience over the last twenty years of pasture-production trials makes one hesitate to quote figures based on anything less than three years' data.

As a method of measuring pasture production from small plots under sheep-grazing the "Sears" technique can hardly be criticized from the point of view of accurate records, providing the treatments are adequately replicated and the common enclosures are "paired," as in "mowing and grazing" trials, so that continuous production records may be obtained. There is only the possibility of an "irrigation" effect from applying the watered-down droppings in dry weather which may affect the comparative and total production figures.

Unfortunately the technique is laborious, time-consuming, and expensive and cannot be carried out on other than experimental areas where large staffs are available. Where it can be carried out, the technique is a very valuable one.

(5) "*Pure White-clover Sward*" Technique

The importance of legumes as "indicator plants" to show fertilizer responses has long been recognized. Statements have been made that it is this element of the sward that responds to dressings of phosphates, calcium, and potash rather than the grasses, which benefit indirectly as a result of the improved vigour and therefore greater nitrogen-fixing power of the clover species. Thus grasses are said to show a response to nitrogen rather than to the other manurial elements when they benefit indirectly from a clover response to fertilizer treatments.

This belief is supported by the following experimental results :—

- (a) Observations and measurements made on mixed swards following the application of fertilizers show that the clovers generally benefit from applications of phosphates, potash, and calcium (where the soil is deficient in available supplies of these minerals), but show no benefit or even retarded growth from the use of nitrogen fertilizers. The opposite is true for the grass species, which show responses to nitrogen shortly after application, but less marked and slower responses to the other elements applied in fertilizers.
- (b) Swards deficient in legumes show little or no response to lime, phosphate, or potash applications, but generally show marked, if temporary, responses to nitrogen.
- (c) Clover swards show more marked responses to lime, phosphates, and potash than do mixed swards (see later).

These factors suggest the use of pure clover swards for measuring responses to fertilizers. While results from such trials could not be directly applied to farming practice, they should be most useful in preliminary work and as a means of ascertaining small differences between treatments. Further, the use of pure clover swards overcomes the necessity to graze the trial with sheep to maintain the sward, and thus the complicating factors associated with sheep-grazing are eliminated. Plots can be of small size and adequately replicated so that results from them may be statistically examined. In addition, the mower gives a representative sample for weighing from each plot and one not subject to the error which exists when tall and close-growing species are associated in the plots to be mown and weighed.

Efforts to establish pure white-clover swards by seeding met with limited success and gave areas of great variability in type and vigour of clover plants present. The present technique, which has proved most satisfactory, is to propagate a single selected plant vegetatively, to plant out the resulting clones on the trial area, and to maintain them in pure association by adequate weeding. Considerable labour in weeding is necessary until the ground is covered by the required clover. It is an advantage to select a clover plant with an easily distinguished marking so that it may be readily separated from volunteer plants.

When mowings are commenced, the clover clippings are discarded after weighing and after samples are taken for analysis. It is proposed in future to replace in the form of artificial fertilizers the nutrients removed by the mown clippings, but even without this refinement the results secured to date are particularly interesting, as is shown by Table VII.

TABLE VII.—YIELDS OF DRY MATTER RELATIVE TO CONTROL (NO TREATMENT) AS 100 :  
RUAKURA

Treatments per Acre.	Mowing Trial "A" under Mowing with Clippings returned (no Grazing, Mixed Sward), (1940-42).	Mowing Trial on Pure White-clover Sward (Clippings removed, no Grazing), (1944-45).
(1) Superphosphate 3 cwt.	119.4	176.7
(2) Superphosphate 3 cwt. plus lime 1 ton initially, 3 cwt. annually	122.8	189.2
(3) As for (2) plus 30 per cent. potash salts 2 cwt.	129.8	210.5

Both these mowing trials were on the same soil type (Horotiu sandy loam) on Ruakura. The difference in response to treatment shows the value this type of trial is likely to have for future work and the importance of the clover elements of the sward. Several additional trials under this technique are in the initial stages, and it is hoped to examine results from these trials with those from other methods of measuring responses.

#### (6) *Additional Methods worthy of Trial*

(a) *Pot Trials.*—Several small-scale investigations have been made in which recognized methods of carrying out pot trials have been employed. These have been made chiefly by Dr. E. B. Davies, Soil Fertility Research Station, Hamilton. It is hoped to expand this work considerably, with particular emphasis on the discovery of the most suitable "indicator plants" (including white clover) and the relationship that results from pot trials show with those from field experiments.

Pot trials, while they will always have a valuable place in grassland research, must be followed up by field experiments, because of the following reasons :—

- (i) Grassland shows extreme variability and sufficiently representative swards are not usually secured in pots. It is necessary to use "indicator plants."
- (ii) The disturbing of the soil profile when filling the pots destroys the physical nature of the soil as it is found in the field. It is possible that techniques may be developed to overcome this drawback.
- (iii) Drainage and water relationships and climatic factors are dissimilar in pots from conditions existing in the field.
- (iv) Leaching of fertilizers and chemical and microbiological soil reactions may differ.
- (v) It is not possible to introduce the effect of the grazing animal.

On the other hand, the pot trial is rapid, economical of space and labour, and, what is most important, can be conducted practically under laboratory control conditions, being independent of soil and climatic variations. The accuracy of measurement is therefore high.

What is required is some factor by means of which differences shown in pot trials may be translated into differences shown in field trials. It is hoped to commence this work at the Soil Fertility Research Station, Hamilton. A promising preliminary line of approach is the use of large pots in which undisturbed soil profiles can be placed.

(b) *The Use of Tethered or "Enclosed" Animals for Grazing.*—One method of avoiding "transference of fertility" which has resulted from animals grazing in a common enclosure is to limit the movement of them across the borders of the plots. This immediately introduces difficulties of grazing, but possibly these difficulties might not be insuperable. Trials were made by Mr. A. W. Hudson at Marton in tethering sheep so that each animal could graze but one plot, but the work was not persisted with because of the difficulties of training the animals to behave normally when tethered. On the other hand, other animals such as goats, cows, or steers may be more easily tethered. It is considered that the method has possibilities which will be worth following up with further trials.

With sheep grazing in small enclosures, each one of which covers a single plot, great care would have to be taken to see that the pastures were not overgrazed. Plots would have to be of sufficient size to give grazing for a single animal for about a day. It is likely that the grazing of such

areas would be uneven until the animals were accustomed to a great deal of handling. Particular care would need to be taken to ensure that the sheep did not "bring-in" or "remove" more nutrients than those represented by the herbage they consumed.

(c) "*Alternate Year*" Trials.—One of the major difficulties which arise when "mowing only" trials are undertaken is the sward change and deterioration which occurs when the selective action of grazing is omitted. With "mowing and grazing" trials this deterioration is prevented by alternating mowing and grazing so that no area is under "mowing only" for more than about six to eight weeks. It is considered that the average sward will not be seriously affected by mowing for a much longer period than this, particularly if the clippings are returned to the plots after cutting. The suggestion is, therefore, that grazings alternate with mowings at yearly intervals, as follows:—

First Year: Area A under "mowing only" (weights secured); area B is prepared for trial.

Second Year: After top-dressing, Area B is under "mowing only" (with production records); area A is grazed (no production records).

Third Year: After top-dressing, Area A is mown (with production records); area B is grazed.

And so on.

By this means no transference of the nutrients applied in the annual top-dressings can occur, though any residual effects of applications will have been affected to some degree by the period under grazing.

This method is at present under trial at the Marton Experimental Area on both a pure white-clover area as well as a mixed sward.

(d) Trials on "*Pure Grass*" Swards.—While there appears to be little doubt that leguminous plants respond more readily to applications of phosphates, potash, and calcium than do the grasses, there is also little doubt that the reverse is true in the case of nitrogen fertilizers. It would therefore seem desirable to establish pure grass swards for trials with such fertilizers, and the practicability of such swards is being investigated at Hamilton.

(e) "*Strips*" out of Paddocks before Grazing.—This method is used to give rough estimates of the herbage production in fields under grazing and can often be used to advantage to supplement stock grazing and production data.

It consists in sampling the field by means of mowing measured strips shortly before the area is grazed. The assumption is made that the grazing will reduce the herbage to the height of mowing so that the weight obtained will give an estimate of the amount of feed available to the stock.

Because of the following defects an accurate figure of total production is not obtained.

- (i) No estimate is made of the production of herbage during the period the trial area is under grazing.
- (ii) It is most difficult to graze a field to the height represented by the mown area, and therefore succeeding estimates of production are high or low according to whether the grazing was above or below this "mower height."
- (iii) Grazing in any case leaves the field with an uneven height of pasture and not one equivalent to an even, mown sward.
- (iv) If the field is small, difficulty will soon be experienced in avoiding previously mown strips. These are prominent for a considerable time usually, because grazing is concentrated on such areas.

This method has largely been replaced by the "enclosure" technique, which is described in the next section.

(f) *Sampling with Shears*.—The mower does not remove herbage in a manner comparable with the grazing animal, and in all techniques in which it is used there is an element of artificiality as a result. Sheep, for instance, will graze closer to the ground than a mower can cut, and they will thrive and obtain feed from a pasture which, according to mower weights, is not yielding anything. It is very difficult to suggest alternatives to the mower. "Plucking" the grass is one method, but here the personal element is too great.

The use of shears, which enable the herbage to be removed at ground level, is one method of overcoming the difficulty. Unfortunately, it is laborious and time consuming and only small plots can be sampled by such means; further, the herbage sample is frequently contaminated with dirt. Once sampled, the area would need to be avoided for a long period.

Such a method does have the advantage that a "trimming" cut is not required, and is also of use in cases where a rough ground surface or one which "pugs" badly with stock makes it difficult or impossible to use a mower. Investigation is being made into suitable types of shears, and it is not unlikely that these can be so designed that the herbage may be cut with comparative ease.

## B. LARGE-SCALE ANIMAL PLUS GRASSLAND PRODUCTION TRIALS

These investigations require considerable areas of ground and are relatively costly and time-consuming to carry out. The policy of the Fields Division has been to use such methods to translate the records obtained from small-plot trials into records of direct application to farming practice. The numbers of comparisons per trial must be small in comparison with those which may be handled with small-plot trials or the experiments become unwieldy.

They may be considered as follows:—

- (1) "Enclosure" technique.
- (2) "Difference" technique.
- (3) "Constant animal weight" technique.
- (4) Animal production and growth trials.

### (1) "Enclosure" Technique

This has proved the most satisfactory method so far adopted and is one which can be used in conjunction with a wide range of stock trials.

In this technique grassland production is measured by means of movable frames or enclosures, the sizes adopted being 11 ft. square, 11 ft. by 5 ft. 6 in., and 5 ft. 6 in. square, according to the size of the experimental field. The technique of measurement is as follows, each measurement figure requiring a pair of frames (A and B) to obtain continuous production records:—

Day.

- 1 .. Frame A is placed in field.
- 10 .. Herbage in frame A (now all above "mower height") is trimmed. Frame B is "placed."
- 20 .. Herbage in frame A is mown and weighed and frame is placed in a new position. Herbage in frame B is trimmed.
- 30 .. Herbage in frame B is mown and weighed. Frame B is placed in a new position in the field. Herbage in frame A is trimmed.

And so on.

By this method continuous production records are secured independently of grazing as long as the fields are under normal grazing management. Weights are secured from areas which have been mown but once previously (the "trimming" cut), and therefore the sward in the enclosure is likely to be very similar in composition to that of the surrounding field.

The grazing management must be such that the pasture is under control at all times. A lengthy growth when trimmed produces a form of recovery growth quite different from that from the sward under normal grazing. In any case, it is not advisable to mow and weigh until at least a week after trimming, as this "recovery growth" grows at a rate quite different from normal growth and frequently has a different composition. Such growth tends to "harden off" in three or four days and become normal in all respects.

A modification of this technique can be used where strict grazing control is possible, and with this modification only half the number of frames is required:—

Day.

1 .. Frame is placed in the paddock.

2-5.. Area is grazed.

5 .. Herbage in frame is trimmed.

10 .. Field is grazed.

15 .. Herbage in frame is mown and weighed. Frame is placed in a new position after trimming the area on which frame is to be placed.

20 .. Field is grazed.

25 .. Herbage in frame is mown and weighed. Frame is placed in a new position after trimming the area on which frame is to be placed.

And so on.

The essential feature of the above method is that sufficient time must elapse between grazing the field and trimming an area in it (at the same time as the herbage protected by the frame is mown and weighed). This procedure is necessary in order that all the grazed herbage may recover to a sufficient height to enable it to be evenly "trimmed" with the mower.

The "enclosure" technique may be applied to a layout of treatments so fenced that each treatment is in a separate field and thus "transference of fertility" from one treatment to another is avoided. With fields of a sufficient size (say one-tenth of an acre or greater for sheep) normal grazing management is possible. In such cases stock-production data (live weight, milk, and wool yields, &c.) and stock-grazing days can form a very useful adjunct to the grassland-production figures as measured by the "enclosure" technique. The full trial of this nature involves the use of holding-paddocks for such stock, each holding-paddock being treated in a manner identical with one of the experimental paddocks. The flocks of animals can, by such means, be maintained solely on each individual treatment and the fullest possible data can be secured thereby under conditions where no transfer of nutrients by stock from one treatment to another is possible.

It is possible to use the "enclosure" technique on small-plot trials using smaller frames. In some cases this adaptation is very useful, as it enables records to be secured independently of the grazing of the trial area. Another adaptation of the method is in "rate of growth" trials, by which means it is possible to study seasonal variations in grassland production. The essential difference from the normal technique, where mowing is carried out only when the pasture is at the usual height of growth for grazing, is the cutting of the sward at *regular* intervals, irrespective of the



growth present. This enables the troughs as well as the peaks of production to be clearly defined. The usual interval is a fortnight, but with overlapping growth periods information of fluctuations in production at smaller time intervals can be secured.

Table VIII gives the percentage standard and sampling errors on two trials at Marton under the "enclosure" technique. It will be seen that these are reduced to a satisfactorily low figure after the initial one or two years. This effect is possibly due to an evening-up of the small experimental fields (of  $\frac{1}{4}$  acre) due to more even grazing and better utilization of the herbage than was the case in the initial paddock before and for a year or two after it was subdivided into experimental paddocks. (NOTE.—The sampling error in each case is estimated from two samples per plot.)

TABLE VIII.—STANDARD ERRORS AND SAMPLING ERRORS OF TRIALS AT MARTON UNDER "ENCLOSURE" TECHNIQUE

Trial.	Errors per Plot as a Percentage of Mean Plot Yield.	
	Standard Error.	Sampling Error.
R. 1940-41.. ..	14.5	7.3
1941-42.. ..	13.6	5.1
1942-43.. ..	4.0	4.2
1943-44.. ..	1.3	2.1
1944-45.. ..	3.7	2.9
S. 1940-41.. ..	11.6	4.0
1941-42.. ..	7.6	4.8
1942-43.. ..	6.8	11.1
1943-44.. ..	4.8	4.8
1944-45.. ..	6.8	8.0

## (2) "Difference" Technique

This is a simple modification of the enclosure technique, but one which enables an estimation to be made of the consumption of herbage by the grazing animal—i.e., it gives a measure of the utilization of pasture growth.

It simply consists in recording the weights of the "trimming" cut, and the amount consumed by the animal is then estimated as follows:—

Day.

- 1 .. Field is grazed. After sheep are removed, areas are mown and weighed and frames placed in position over them. (Suppose the weight of the clippings from this cut is  $\frac{1}{2}$  lb.)
- 10 .. Field is grazed.
- 11 .. After sheep are removed—
  - (a) Mow and weigh herbage enclosed by frames (say this amounts to 5 lb.).
  - (b) Mow and weigh adjacent areas each of the size enclosed by the frame (say the clippings from these weigh 1 lb.). Place the frames on these trimmed areas.

And so on.

Then—

- (a) The production of herbage for the period day 1 to day 11 is 5 lb.
- (b) The amount of herbage eaten at the grazing-days 10 and 11 is the herbage remaining after the previous grazing ( $\frac{1}{2}$  lb.) *plus* the production in the growth period (5 lb.) *minus* the herbage remaining after the second grazing (1 lb.)—i.e.,  $4\frac{1}{2}$  lb.

This technique suffers from the inaccuracy in that sheep (and some other animals) graze closer to the ground than the mower can cut and therefore the weight of trimmings is liable to be on the low side, as is the estimate of consumption and production. Relative figures should be reasonably comparable, however, and Table IX, which is based on a trial at Marton conducted under this technique, shows that reasonably consistent figures may be obtained when using it. The fields in this trial were sown to different pasture mixtures and under different managerial conditions. Nevertheless, the measure of the consumption of herbage by sheep in each field is reasonably constant.

TABLE IX.—CONSUMPTION OF HERBAGE BY SHEEP (AS MEASURED BY THE  
"DIFFERENCE" TECHNIQUE): TRIAL O, MARTON  
(Period 2nd May, 1944, to 1st May, 1945)

Field No.	Herbage consumed per Sheep per Day.			
	Green Material.		Dry Matter.	
	May to November, 1944.	November, 1944, to May, 1945.	May to November, 1944.	November, 1944, to May, 1945.
1 .. .. .	12.9	19.4	2.36	3.49
2 .. .. .	12.6	18.9	2.30	3.43
3 .. .. .	13.2	22.6	2.36	3.86
4 .. .. .	12.9	20.5	2.28	3.79
5 .. .. .	10.1	19.2	2.25	3.70
6 (summer spelled) .. .. .	13.0	..	2.36	..
7 (winter spelled) .. .. .	..	22.8	..	3.90
Mean .. .	12.5	20.6	2.32	3.70

The variation between the two periods as shown in Table IX is, of course, the reflection of the difference in amount of pasture available as well as the higher productive ration needed by the sheep in the summer period.

### (3) "Constant Animal Weight" Technique

This technique appears to have been developed by Rhoad and Carr(3) using mature steers, and preliminary work which has been carried out at the Soil Fertility Research Station, Hamilton, indicates that the method can be used successfully with dry sheep.

Essentially the technique aims at keeping the experimental animals at a constant weight. Differences between the various experimental pastures can then be measured directly in terms of carrying-capacity of such animals. There are two methods of doing this:—

- Altering the numbers of stock in the experimental fields on the basis of changes in their weight. If slight gains in weight are recorded during the weighing period (usually a week), additional stock numbers will be put into the field concerned until the animals are on a maintenance diet. Conversely, if they are losing weight, the stock numbers have to be reduced.
- Altering the size of the field in which the animals are grazed so that their weight is kept constant. This procedure introduces variation in treatment of the experimental fields and is therefore considered not as desirable as the alternative method.

Obviously considerable experience in using this technique and previous investigation into the reaction of the animals used are essential, but it does offer a means of obtaining data directly in the terms in which it is required—i.e., those of the animal. Mature dry animals must be used, and to some extent this limits the application of the method. The demands of a milking-cow are probably quite different from those of a mature steer on a maintenance ration, and important questions such as palatability and efficiency of pasture utilization are unlikely to be satisfactorily translated from one type of stock to the other. Nevertheless, there are a great many problems to which this technique appears to offer promise as a means of obtaining satisfactory solutions.

#### (4) *Animal Production and Growth Trials*

Because trials of this nature represent data in the form in which it is of immediate practical application, they are usually considered as the final stage of any research problem dealing with grassland. Unfortunately, they are costly and take up large areas of land, while needing much specialized labour for a few comparisons. It is therefore necessary to reduce the number of comparisons down to the smallest possible figure by methods such as small-plot grass trials before attempting this type of experiment.

As with all experiments involving the use of stock, the variability of the animal introduces another and most difficult factor into the comparisons. The use of identical twins (which is being investigated by McMeekan at the Ruakura Animal Research Station, Hamilton) is a promising means of overcoming this difficulty, but such animals are very difficult to acquire.

No attempt will be made to explain the many types of trial of this nature, except to mention an alternative line of approach, and that is the testing out of promising treatments on a farm scale. Such a method does not give accurate data perhaps, but it offers practical demonstration of the feasibility of the treatment in question. In the long-run this is the method which finally decides its adoption into farming practice, and in many instances it is not necessary to introduce the large-scale animal trial at all, progressive farmers having demonstrated the usefulness or otherwise of the suggested treatments on their own property.

When trials of this nature are undertaken they should always be run in conjunction with pasture-production measurements, and the "enclosure" technique, in the opinion of the writer, is of great use in this connection.

#### C. DEMONSTRATIONAL STOCK-GRAZING TRIALS AND OBSERVATIONAL TRIALS

These trials are used extensively in connection with the extension work of the Fields Division, and in most cases they have two main functions:—

- (a) *Demonstrational*.—As a means of demonstrating to the farmer methods or treatments developed at research institutes or experimental stations these trials are invaluable. Perhaps pasture species and strains trials have proved to be the most useful type of demonstrational trial.
- (b) *Experimental*.—Although under observation only, trained observers can give comparisons on which considerable reliance may be placed. Large areas of land can be quickly surveyed by this type of trial, which has its greatest application in observational pasture top-dressing experiments, hundreds of which have been laid down throughout New Zealand.

Observational trials of this nature on grassland usually consist of a series of small plots (20 × 25 links is a standard size), and these are reported on at regular intervals. Stock-grazing trials are usually on the "half-paddock" system, whereby a uniform field is divided into two halves, each half being treated according to the comparison desired. Observations on pasture growth from these half-paddocks are supplemented by stock-carrying figures, which are supplied by the co-operating farmer.

All these trials are laid down on farms and under normal farming conditions, with the co-operation of the farmer concerned. They serve, therefore, as a direct link between research and the farmer as well as having a considerable value on their own account as field experiments.

### CONCLUSIONS

This survey demonstrates the need for improved techniques in grassland research. Despite this need, however, it is considered that considerable progress has been made, and this appears likely to be continued in the near future.

Some of the techniques previously extensively used by the Fields Division have been the subject of considerable criticisms. This applies particularly to the "mowing and grazing" technique, and although the limitations of this method are fully realized, the writer has endeavoured to meet some of the criticisms and to show that the results secured using the technique are not invalid. With an appreciation of its limitations, it is considered that the "mowing and grazing" technique may still be usefully used in grassland research.

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## THE INFLUENCE OF CERTAIN MILKING-MACHINE ADJUSTMENTS ON THE RATE OF MACHINE-MILKING

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### Summary

Cows were milked under strictly controlled milking-conditions and the milking process measured by means of an automatic milk-flow-recording apparatus. Using the average rate of flow given by the milk-flow curves as a criterion, the effect of the following machine-milking factors was examined.

Rate of application of the squeezing and releasing action of the pulsator : pulsator speeds of 21, 42, and 84 pulsations per minute at a vacuum of  $14\frac{1}{2}$  in. and vacuums of 10 in. ;  $14\frac{1}{2}$  in. ; and 19 in. at a pulsator speed of 42 pulsations per minute. Generally, no significant difference in milking rate was found between the different sets of conditions.

In a previous paper(1) the influence of the level of stimulus applied by the milking-machine pulsator on the rate of milking was described. A subsequent paper(2) describes the characteristics of the milk-ejection curve of normal cows under strict milking-conditions. The present paper gives the results of experiments which cover factors other than the pulsator. The milking-conditions were, unless otherwise mentioned, as described in the second paper referred to above(2). Briefly, they were as follows : A single-bail orthodox releaser plant with slide pulsators was operated at a pulsator rate of 42 pulsations per minute and ratio of 50 : 50. The vacuum was  $14\frac{1}{2}$  in. A constant tension was maintained on soft rubber topped inflations.

The cows were leg-roped and the udders washed immediately prior to application of the cups. For the first experiment the cows were machine-stripped by pulling down the cups when the milk-flow rate as indicated by flow-measuring sight-glass(2) fell below 0.5 lb. per minute. When the flow had fallen again the cups were removed.

The measurements given below were obtained by the use of the recording apparatus previously described(3). All recordings were made at afternoon milkings. In addition, on days when recordings were not being made, two time-clocks (one for each group), operated by relays which closed while the cups were on the cows, were used to obtain total milking-times. The clocks gave an accurate total time over a period of hours but could not be read in small enough units to enable the variance of the individual times to be determined. The average figures given are useful when taken in conjunction with the results from the recording-apparatus.

The experiments to be described were carried out on a group of twelve cows milked under strictly controlled conditions for a period and milk-ejection curves determined. The cows were paired on the basis of similarity of milk-ejection curves.

Generally, the average rate of milking in pounds per minute is taken as a criterion in the comparison of treatments.

The first experiment was designed to discover whether the rate of application of "squeeze" and "release" by the pulsator would affect milking-rate. This is another aspect of the problem of the effect of pulsator stimulus.

One cow of each pair was milked with normal pulsation during one period of the experiment, the other cow being milked with a pulsator adjusted so that during the squeeze phase the pulsator chambers of the teat-cups did not quite reach atmospheric pressure. During the second period the groups were changed over. The pulsator vacuum graphs are shown in Fig. '1. The results of the experiment are set out in Table I.

The average milk-flow rate (W/t) was measured from the milk-flow curve.

TABLE I

Date.	Control.	Experimental.	Date.	Control.	Experimental.	Date.	Control	Experimental.
No. of Cow ..	39.	X4.	No. of Cow	O4.	X80.	No. of Cow	121.	X277.
13/11/45 ..	3.1	..	13/11 ..	7.9	4.3	13/11 ..	2.7	2.7
15/11 ..	1.8	4.5	15/11 ..	2.0	2.4	15/11 ..	3.2	2.4
19/11 ..	1.9	..	19/11 ..	5.1	5.7	19/11 ..	3.0	3.3
23/11 ..	2.4	3.9	23/11 ..	3.3	3.0	23/11 ..	2.9	2.5
26/11 ..	1.7	4.4	26/11 ..	2.9	4.8	26/11 ..	3.4	2.3
No. of Cow ..	X4.	39.	No. of Cow	X80.	O4.	No. of Cow	X277.	121.
27/11 ..	4.2	2.0	27/11 ..	2.8	3.4	27/11 ..	2.7	2.8
30/11 ..	4.7	1.4	30/11 ..	1.8	2.9	30/11 ..	3.3	3.1
1/12 ..	3.6	1.8	1/12 ..	3.6	8.1	1/12 ..	2.5	3.1
7/12 ..	4.0	1.6	7/12 ..	4.3	3.4	7/12 ..	2.9	2.5
10/12 ..	3.4	2.9	10/12 ..	4.1	3.3	10/12 ..	2.8	3.4
No. of Cow ..	X66.	X18.	No. of Cow	167.	142.	No. of Cow	186.	180.
13/11 ..	3.8	4.9	13/11 ..	2.2	2.1	13/11 ..	3.1	3.2
15/11 ..	4.0	4.8	15/11 ..	2.3	1.8	15/11 ..	2.6	3.1
19/11 ..	3.8	4.9	19/11 ..	1.9	1.8	19/11 ..	3.2	2.9
23/11 ..	3.8	5.1	23/11 ..	2.2	2.6	23/11 ..	2.8	2.8
26/11 ..	3.7	4.4	26/11 ..	2.0	1.7	26/11 ..	2.7	4.2
No. of Cow ..	X18.	X66.	No. of Cow	142.	167.	No. of Cow	180.	186.
27/11 ..	4.8	4.5	27/11 ..	2.1	2.6	27/11 ..	3.6	3.0
30/11 ..	4.7	4.4	30/11 ..	2.0	2.7	30/11 ..	3.6	3.4
1/12 ..	4.6	4.2	1/12 ..	1.9	2.6	1/12 ..	3.7	2.2
7/12 ..	4.5	5.1	7/12 ..	1.6	2.2	7/12 ..	3.2	2.8
10/12 ..	5.9	4.6	10/12 ..	1.8	2.2	10/12 ..	3.3	2.5

These results may be summarized as follows :—

Mean milking-rate for control treatment .. = 3.25 lb./minute.

Mean milking-rate for experimental treatment = 3.37 lb./minute.

Difference .. .. = 0.12 lb./minute.

This difference is not statistically significant.

The difference between the treatments is not significant.

The time-clocks gave the following figures :—

TABLE II.—MILKING-TIMES, IN MINUTES

Period.				Control.	Slow Squeeze Group.
1	..	..	..	7.57	8.52
2	..	..	..	7.68	6.60
Means .. ..				7.63	7.56

Again it is evident that there is little difference between the groups.

As the cows were "machine-stripped" by pulling down on the teat-cups throughout this experiment, the following figures are of interest.

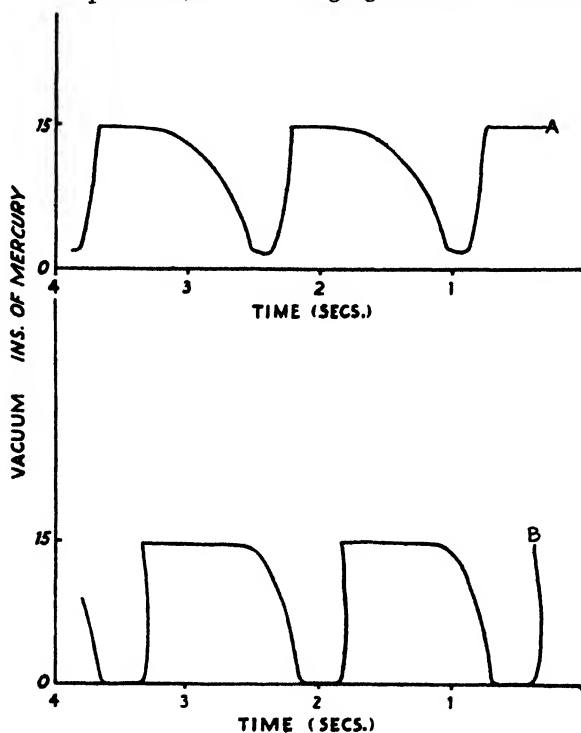


FIG. 1.

The average milking-time as indicated by the clocks was 7.60 minutes. This figure was governed by the flow-indicating sight-glass, and also included both morning and evening milkings. This accounts for the fact that the figure is larger than the average time given by the milk-flow-recording device, which was 6.67 minutes. When records were being made the machine-stripping and removal of the cups were controlled by an estimate of the gradient of the milk-ejection curve, which is a more precise method than the sight glass. If the time taken to "machine-strip" the cows is deducted from the over-all time as given by the milk-ejection curves, we get a figure of 4.59 minutes. This machine-stripping time of 2.08 minutes supports the contention of many practical farmers that machine-stripping is not much, if any, faster than hand-stripping. The average amount of machine-strippings for the period was 0.82 lb. of milk—i.e., 5.8 per cent.

Throughout subsequent experiments machine-stripping was abandoned and a complete non-stripping schedule adopted.

The second experiment made a comparison between milking-rates for cows milked with normal pulsation and those milked using a pulsator giving a normal form of pulsator vacuum curve but double the number of pulsations per minute. Figure 2 shows the normal and double pulsator vacuum graphs.

Table III gives the results of the experiment.

TABLE III

Date.	Control.	Experi- mental.	Date.	Control.	Experi- mental.	Date.	Control.	Experi- mental.
No. of Cow ..	39.	X4.	No. of Cow	O4.	X80.	No. of Cow	121.	X277.
15/1/46 ..	2.3	1.9	15/1 ..	4.9	2.5	15/1 ..	2.6	2.2
18/1 ..	1.0	1.6	18/1 ..	1.2	2.9	18/1 ..	3.3	1.3
21/1 ..	1.1	1.8	21/1 ..	1.4	3.1	21/1 ..	2.6	2.9
No. of Cow ..	X4.	39.	No. of Cow	X80.	O4.	No. of Cow	X277.	121.
22/1 ..	2.1	1.0	22/1 ..	2.7	1.6	22/1 ..	2.2	2.7
25/1 ..	..	..	25/1 ..	1.5	0.7	25/1 ..	1.5	2.9
28/1 ..	2.2	0.9	28/1 ..	1.5	1.3	28/1 ..	2.8	2.3
No. of Cow ..	X66.	X18.	No. of Cow	167.	142.	No. of Cow	186.	180.
15/1 ..	2.9	3.8	15/1 ..	2.1	1.9	15/1 ..	1.4	2.2
18/1 ..	3.0	3.4	18/1 ..	2.2	2.4	18/1 ..	2.0	3.0
21/1 ..	4.2	3.5	21/1 ..	2.3	1.9	21/1 ..	2.5	4.8
No. of Cow ..	X18.	X66.	No. of Cow	142.	167.	No. of Cow	180.	186.
22/1 ..	5.3	2.8	22/1 ..	2.0	2.8	22/1 ..	1.6	2.4
25/1 ..	4.4	2.2	25/1 ..	1.5	2.2	25/1 ..	2.7	2.5
28/1 ..	2.8	2.8	28/1 ..	1.7	2.4	28/1 ..	2.2	1.9

These results may be summarized as follows :—

Mean milking-rate for control treatment .. = 2.38 lb./minute.

Mean milking-rate for experimental treatment = 2.32 lb./minute.

Difference .. .. = 0.06 lb./minute.

This difference is not statistically significant.

Table IV gives the results indicated by the time-clocks.

TABLE IV

Period.				Time, in Minutes.	
				Control.	Double Pulsator Speed.
1	..	..	..	4.82	5.18
2	..	..	..	4.82	4.82
Means				4.82	5.00



These figures confirm the previous conclusion. It is interesting to note the faster over-all milking-rate during this second experiment when machine-stripping was abandoned.

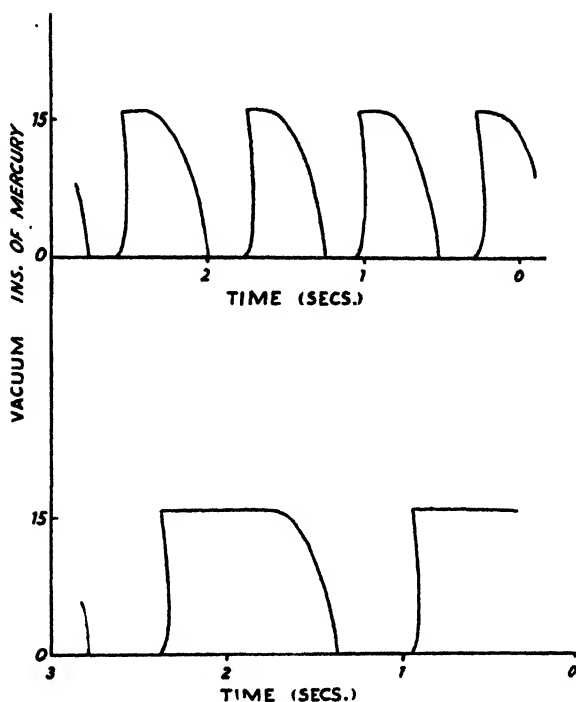


FIG. 2.

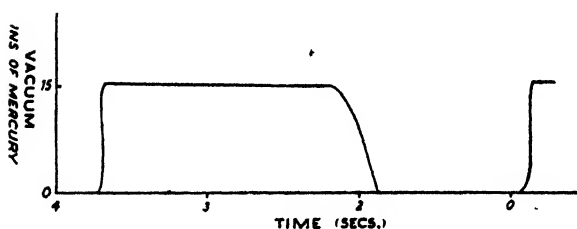


FIG. 3.

The only difference between the two groups noticeable to the milker was the tendency for the teat-cups to fall more readily from the cows when the double pulsation rate was in use. This may be due to the fact that the faster pulsation would consume more air and so reduce the reserve capacity of the vacuum-pump. This is a real argument against fast pulsation.

The third experiment was identical with the second, except that the experimental groups were milked at one-half the normal pulsation speed. The pulsation vacuum graph is shown in Fig. 3. The results are set out in Table V.

TABLE V

Date.	Control.	Experi- mental.	Date.	Control.	Experi- mental.	Date.	Control.	Experi- mental.
No. of Cow ..	39.	X4.	No. of Cow	O4.	X80.	No. of Cow	121.	X277.
29/1/46 ..	..	2.1	29/1 ..	1.6	2.1	29/1 ..	1.6	2.3
1/2 ..	0.9	1.8	1/2 ..	1.5	1.5	1/2 ..	1.9	1.5
4/2 ..	1.2	1.2	4/2 ..	1.4	2.9	4/2 ..	2.4	1.2
No. of Cow ..	X4.	39.	No. of Cow	X80.	O4	No. of Cow	X277.	121.
5/2 ..	2.0	0.7	5/2 ..	1.2	1.8	5/2 ..	2.1	1.8
8/2 ..	1.4	2.0	8/2 ..	2.1	1.5	8/2 ..	1.6	2.1
11/2 ..	..	0.7	11/2 ..	0.5	1.5	11/2 ..	2.9	2.4
No. of Cow ..	X66.	X18.	No. of Cow	167.	142.	No. of Cow	186.	180.
29/1 ..	3.0	2.4	29/1 ..	1.7	2.1	29/1 ..	2.1	2.1
1/2 ..	3.1	2.7	1/2 ..	1.6	1.5	1/2 ..	2.3	1.6
4/2 ..	1.8	2.3	4/2 ..	2.3	1.4	4/2 ..	2.3	1.0
No. of Cow ..	X18.	X66.	No. of Cow	142.	167.	No. of Cow	180	186
5/2 ..	3.4	3.7	5/2 ..	0.8	1.5	5/2 ..	1.3	1.7
8/2 ..	2.8	2.2	8/2 ..	1.4	2.0	8/2 ..	3.2	2.0
11/2 ..	2.5	1.8	11/2 ..	1.5	2.3	11/2 ..	1.4	2.0

These results may be summarized as follows:—

Mean milking-rate for control treatment .. — 1.88 lb./minute.

Mean milking-rate for experimental treatment = 1.88 lb./minute.

Difference .. Nil.

There is no significant difference.

Table VI gives the time-clock readings:—

TABLE VI

Period.				Time, in Minutes.	
				Control.	Half Pulsator. Speed.
1	..	..	..	4.82	4.90
2	..	..	..	4.91	5.17
Means .. ..				4.87	5.04

Again we find a small difference. In comparing these figures with those in Table IV it is of interest to note that both double and half-normal pulsator speeds give slightly slower milking than the normal group. It is clear that quite large variations in pulsator speed have no significant effect on milking-rate.

The following two experiments are intended to ascertain the influence of level of vacuum on milking-rate under the conditions described earlier. In the fourth experiment the cows are milked at 10 in. and 14½ in. respectively, using the change-over technique applied to the previous experiments. The fifth experiment used vacuums of 14½ in. and 19 in. The vacuum was adjusted and maintained constant by the use of a weighted relief valve with a variable weight.

The results of the experiment with 10 in. and 14½ in. vacuums are set out in Table VII.

TABLE VII

Date.	14½ in. Vacuum.	10 in. Vacuum.	Date.	14½ in. Vacuum.	10 in. Vacuum.	Date.	14½ in. Vacuum.	10 in. Vacuum.
No. of Cow ..	X4.	39.	No. of Cow	X80.	O4.	No. of Cow	X277.	121.
18/12/45 ..	3.2	1.2	18/12 ..	1.9	2.2	18/12 ..	2.1	2.3
21/12 ..	2.8	1.2	21/12 ..	2.5	3.7	21/12 ..	2.5	1.7
24/12 ..	3.3	2.2	24/12 ..	2.6	3.4	24/12 ..	2.6	3.3
No. of Cow ..	39.	X4.	No. of Cow	O4.	X80.	No. of Cow	121.	X277.
25/12 ..	1.7	1.9	25/12 ..	2.5	3.1	25/12 ..	3.0	1.3
28/12 ..	1.7	2.3	28/12 ..	2.4	2.5	28/12 ..	2.6	2.0
31/12 ..	1.4	2.0	31/12 ..	2.5	3.0	31/12 ..	3.3	2.0
No. of Cow ..	X18.	X66.	No. of Cow	142.	167.	No. of Cow	180.	186.
18/12 ..	4.3	4.0	18/12 ..	1.8	2.2	18/12 ..	2.5	2.2
21/12 ..	4.4	2.9	21/12 ..	2.1	1.6	21/12 ..	1.0	1.8
24/12 ..	4.7	3.4	24/12 ..	1.5	1.7	24/12 ..	2.6	2.0
No. of Cow ..	X66.	X18.	No. of Cow	167.	142.	No. of Cow	186.	180.
25/12 ..	3.5	3.5	25/12 ..	1.8	0.98	25/12 ..	2.6	1.6
28/12 ..	4.4	5.5	28/12 ..	1.8	1.3	28/12 ..	2.8	2.6
31/12 ..	3.4	..	31/12 ..	2.5	1.3	31/12 ..	2.6	2.5

These results may be summarized as follows :—

Mean milking-rate for control treatment .. = 2.64 lb./minute.

Mean milking-rate for experimental treatment = 2.42 lb./minute.

Difference .. .. = 0.22 lb./minute.

This difference is not statistically significant.

The time-clock readings are set out in Table VIII.

TABLE VIII

Period.					Time, in Minutes.	
					14½ in. Vacuum.	10 in. Vacuum.
1	..	..	..	..	6.00	7.08
2	..	..	..	..	5.40	5.88
Means	..	..	..	..	5.70	6.48

Table IX gives the results obtained when 14½ in. and 19 in. vacuums were used.

TABLE IX

Date.	14½ in. Vacuum.	19 in. Vacuum.	Date.	14½ in. Vacuum.	19 in. Vacuum.	Date.	14½ in. Vacuum.	19 in. Vacuum.
No. of Cow ..	39.	X4.	No. of Cow	O4.	X80.	No. of Cow	121.	X277.
1/1/46 ..	1.3	2.6	1/1 ..	3.4	3.2	1/1 ..	3.8	3.3
6/1 ..	2.2	2.8	6/1 ..	2.6	2.6	6/1 ..	2.5	2.3
7/1 ..	3.2	2.1	7/1 ..	2.7	1.9	7/1 ..	2.6	3.5
No. of Cow ..	X4.	39.	No. of Cow	X80.	O4.	No. of Cow	X277.	121.
8/1 ..	2.1	2.2	8/1 ..	2.0	1.4	8/1 ..	3.5	2.9
11/1 ..	1.4	1.8	11/1 ..	3.9	1.4	11/1 ..	2.4	3.3
14/1 ..	2.0	1.3	14/1 ..	3.0	2.4	14/1 ..	1.3	2.4
No. of Cow ..	X66.	X18.	No. of Cow	167.	142.	No. of Cow	186.	180.
1/1 ..	2.7	4.1	1/1 ..	2.1	2.3	1/1 ..	3.7	2.2
6/1 ..	5.1	3.9	6/1 ..	3.1	2.1	6/1 ..	2.9	1.3
7/1 ..	4.2	6.2	7/1 ..	1.0	1.7	7/1 ..	3.0	1.9
No. of Cow ..	X18.	X66.	No. of Cow	142.	167.	No. of Cow	180.	186.
8/1 ..	4.3	2.5	8/1 ..	2.0	1.7	8/1 ..	2.3	2.9
11/1 ..	4.4	4.1	11/1 ..	1.9	2.9	11/1 ..	2.5	3.2
14/1 ..	6.0	5.7	14/1 ..	1.8	2.5	14/1 ..	1.9	2.1

These results may be summarized as follows :—

Mean milking-rate for control treatment .. = 2.74 lb./minute.  
Mean milking-rate for experimental treatment = 2.69 lb./minute.  
Difference .. .. = 0.05 lb./minute.

This difference is not statistically significant.

Table X gives the time-clock readings.

TABLE X

Period.				Time, in Minutes.	
				14½ in. Vacuum.	19 in. Vacuum.
1	..	..	..	5.20	5.32
2	..	..	..	5.32	5.11
Means				5.26	5.22

It would appear from the above results that over a period the level of vacuum applied to the cows by a milking-machine does not significantly affect the milking-rate between the limits of 10 in. and 19 in. of mercury.

On the 10 in. vacuum, trouble was experienced in keeping the teat-cups on the cows. This would mean an increase in total milking-time, but as the recorder and clocks automatically stop when the cups fall off the effect would not show in our figures. When the cows were milked by the 19 in. vacuum there were definite signs of discomfort. In order to see whether this effect in any way affected the letting-down of the milk the time in minutes taken for the first 0.54 lb. of milk to be delivered was measured. Table XI gives the results.

TABLE XI

Date.	14½ in. Vacuum.	19 in. Vacuum.	Date.	14½ in. Vacuum.	19 in. Vacuum.	Date.	14½ in. Vacuum.	19 in. Vacuum.
No. of Cow ..	39.	X4.	No. of Cow	O4.	X80.	No. of Cow	121.	X277.
1/1/46 ..	0.3	0.3	1/1 ..	0.4	0.2	1/1 ..	0.7	0.5
6/1 ..	0.2	0.5	6/1 ..	0.3	0.4	6/1 ..	0.4	0.5
7/1 ..	0.2	0.6	7/1 ..	0.2	0.4	7/1 ..	0.4	0.2
No. of Cow ..	X4.	39.	No. of Cow	X80.	O4.	No. of Cow	X277.	121.
8/1 ..	0.8	0.3	8/1 ..	0.2	0.5	8/1 ..	0.4	0.6
11/1 ..	1.0	0.2	11/1 ..	0.3	0.3	11/1 ..	0.3	0.5
14/1 ..	0.2	0.3	14/1 ..	0.5	0.4	14/1 ..	0.4	1.1
No. of Cow ..	X66.	X18.	No. of Cow	167.	142.	No. of Cow	186.	180.
1/1 ..	0.6	0.3	1/1 ..	0.7	0.6	1/1 ..	0.3	0.5
6/1 ..	0.4	0.3	6/1 ..	0.3	0.5	6/1 ..	0.5	0.3
7/1 ..	0.3	0.2	7/1 ..	0.5	1.8	7/1 ..	0.5	0.6
No. of Cow	X18.	X66.	No. of Cow	142.	167.	No. of Cow	180.	186.
8/1 ..	0.3	0.2	8/1 ..	1.1	0.4	8/1 ..	0.8	0.6
11/1 ..	0.2	0.5	11/1 ..	0.6	0.6	11/1 ..	0.6	0.6
14/1 ..	0.2	0.2	14/1 ..	0.4	0.3	14/1 ..	0.5	0.5

These results may be summarized as follows :—

Mean let-down-time for control treatment .. = 0.444 minutes.

Mean let-down-time for experimental treatment = 0.467 minutes.

Difference .. .. = 0.023 minutes.

This difference is not statistically significant.

Evidently the effect of a high vacuum, though causing discomfort, does not result in the cows being slow to let down their milk.

Our results do not agree with those of Smith and Petersen(4), who find that higher vacuums are associated in a non-linear manner with higher milking-rates. No explanation is offered at present. The negative results of our experiments are somewhat surprising in the light of generally held beliefs, although in the field we have not found any marked association between fast milking and high vacuum.

The effect of the different levels of vacuum on one of our cows is of some interest. This cow is a "tough milker" whose average milk-flow rate is consistently low. The milk-flow rates for this cow during the vacuum experiment are set out in Table XII.

TABLE XII

Vacuum.	10.	14½.	19.
	A.	A.	B.
Milk-flow rate, lb./min. . .	1.0	1.8	2.0
" " " " " " " " " " " "	1.3	2.1	1.9
" " " " " " " " " " " "	1.3	1.5	1.9
Means . . .	1.2	1.8	2.0

The difference between the curves for the 10 in. and 14½ in. groups reaches significance, while that for the 14½ in. and 19 in. groups does not.

In Fig. 4 we have set out tracings of the milk-flow curves given by this cow under the three vacuums. In the case of this cow there is a noticeable relation between flow-rate and vacuum. Generally, however, this does not apply to the remainder of the cows.

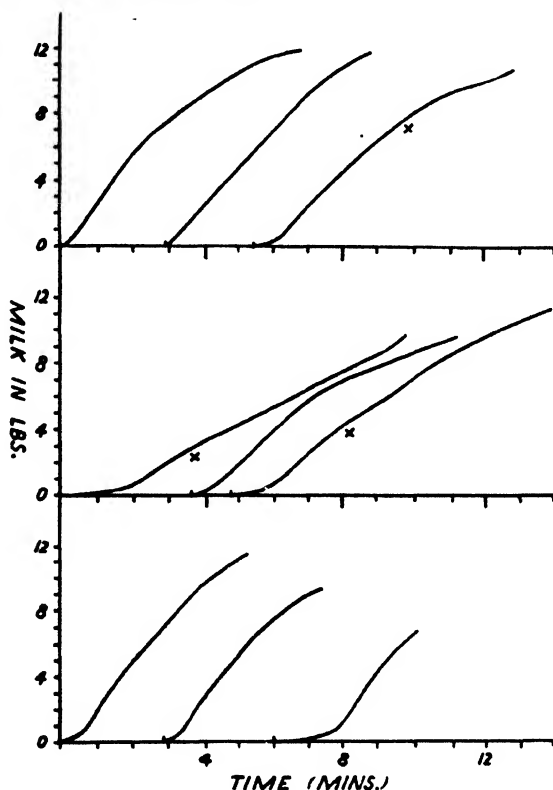


FIG. 4.

#### GENERAL DISCUSSION

It is apparent from the foregoing experimental results that the cow is generally the limiting factor in the milking process. The pulsator may give a "snappy" or a sluggish squeezing action, it may run at 21, 42, or 84 pulsations per minute, the vacuum may be 10 in., 15 in., or 19 in. of mercury,

and there is no significant change in the machine-milking process. With the number of determinations of milk-flow rate at our disposal the experiments are not of a high order of precision. It is likely that with larger numbers of trials some differences may become significant. However, at present we are concerned only with points of interest to the farmer. Finer differences which may throw light on the mechanism of milk let-down are the object of special study. These findings are a surprise when viewed from the point of view of present-day beliefs amongst the milking-machine experts. However, they are not quite so surprising if regarded in the light of the extraordinary variety and condition of machines found in the field which actually milked satisfactorily. The results set out in this paper confirm our earlier conclusion: that shed routine is more important than the mechanical characteristics of the milking-machine.

#### ACKNOWLEDGMENT

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#### APPENDIX

##### STATISTICAL NOTE

An analysis of variance was made on each set of data. For this purpose all cows receiving the control treatment during the first period were placed in Group A, all those receiving the control treatment during the second period in Group B. The distribution of variance between different factors was very similar in all cases, and the result from Table IX only is quoted here as an example. Table I was slightly different as one pair of cows had to be omitted because of a large number of missing values.

*Analysis of Variance of Table IX*

Variance due to—	Degrees of Freedom.	Sum of Squares.	Variance.
Periods .. .. .	1	0.08	0.08
Groups .. .. .	1	0.15	0.15
Cows within Group A or Group B ..	10	153.13	15.31
Treatments .. .. .	1	0.08	0.08
Error .. .. .	10	13.20	1.32
Total .. .. .	23	166.64	7.25

It can be seen that the variance due to individual cows is very high, while that due to groups is low, because of good pairing, although there were large differences between pairs.

The variance for "error" is the sum of the interactions of periods  $\times$  cows within a group. In only one case is this even as low as the variance for treatments, indicating that the non-significance of the treatment differences cannot be attributed to a lack of accuracy in obtaining the data.

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